SCIENCE LIBRARY GENERAL DEC 15 1920 UNIV. OF MICH.

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXIX AUGUST, 1920 NO. 2

CONTENTS

		PAGE
DODDS, GIDEON S.	*Entomostraca and Life Zones*	89
CROZIER, W. J.	*Notes on Some Problems of Adaptation, I* . . .	108
CROZIER, W. J.	*Notes on Some Problems of Adaptation, II* . . .	116
CROZIER, W. J.	*Notes on Some Problems of Adaptation, III* . . .	130
GLASER, R. W.	*Biological Studies on Intracellular Bacteria, No. 1*	133

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor, the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXIX. *August, 1920.* *No. 2.*

BIOLOGICAL BULLETIN

ENTOMOSTRACA AND LIFE ZONES.

A Study of Distribution in the Colorado Rockies.

GIDEON S. DODDS,

West Virginia University.

I. The Region Covered.

In a former paper ('17) I described the altitudinal distribution of the 71 species of Entomostraca known to occur within the limits of the state of Colorado. Fifty-five of these species are from my own collections made in a definite attempt to gather a reasonable amount of information about the nature of the entomostracan fauna at different elevations in this part of the Rocky Mountains. The material included 280 vials of plankton from 124 lakes and ponds at elevations ranging from 4,100 to 12,188 feet above sea level. By far the greater number of these collections are from an area covering adjacent parts of the counties of Boulder, Jefferson, Gilpin, Clear Creek, and Grand, and includes, within a distance of 25 miles, portions of all the life zones from the Upper Sonoran to the Arctic-Alpine. A full description of the topography and climate of this region is included in my former paper. In addition to my own collections from this region, together with a few from other parts of the plains of the state, I have made use of all available records of species collected by others within the state.

The state of Colorado affords an area for study which is not wholly an arbitrary or unnatural one. Its higher portions include the greatest elevations of the Rocky Mountains, with their southern extension of climatic and biotic characters from the north, and its lower portions are typical of the arid and hot conditions of the southwestern portion of the United States, while its position astride the Continental Divide makes it the meeting place of

eastern and western species. Its biota is accordingly composed of a mixture of northern and southern, of eastern and western forms. Reports on plants, birds, and mammals testify to the richness and variety of life here where so diverse conditions are condensed within a restricted area.

In my former paper I referred the Entomostraca of my list to

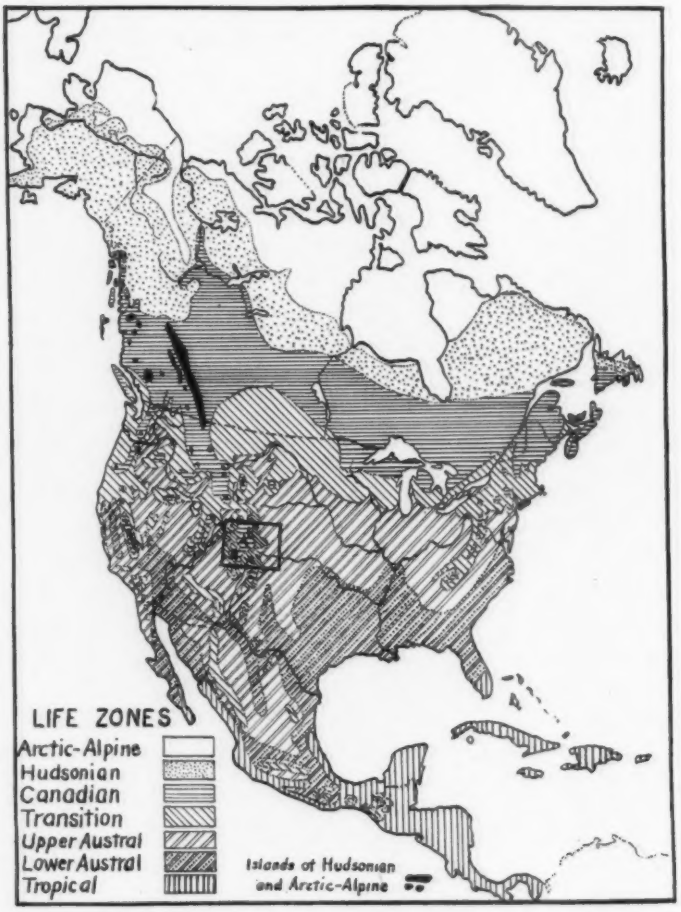

FIG. 1. Life-zone map of North America with outline of state of Colorado drawn in. After Merrian, etc., from A. O. U. Check-list of North American Birds, 3d edition (revised), 1910.

the modification of Schimper's zones adopted by Ramaley in his studies of the vegetation of the area. It seems desirable, however, to tie this fauna to the larger life zones which apply throughout the continent as a whole and which have been mapped out by Merriam and are now made use of by a considerable number of those interested in problems of distribution. Figure 1 shows how these zones are distributed over the continent. Of special interest are the southward extensions and islands of the northern zones over the higher areas, as in the Appalachians, Rockies, and Sierras. The outline of Colorado, indicated by the

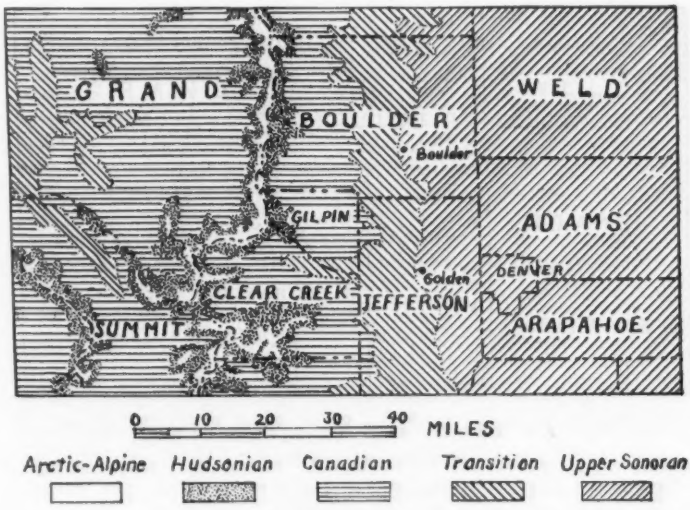

FIG. 2. Life-zone map of the portion of Colorado where most of the writer's collections were made. Shows close approach up Upper Sonoran and Arctic-Alpine Zones and narrowness of other zones in this region. After Cary.

black rectangle, shows the position of the area under consideration with reference to one of these southward extensions. Cary ('11) publishes a more detailed life-zone map of the state, a small portion of which is reproduced in Fig. 2. This includes the area from which most of my records come and shows that within a few miles are met Upper Sonoran, Transition, Canadian, Hudsonian, and Arctic-Alpine Zones.

The approximate altitudinal limits and outstanding floral characters of these zones in this part of the state as given by Cary and verified by my own observations, are as follows:

1. *Upper Sonoran* (arid, western portion of Upper Austral): Western edge of the Great Plains as far as the base of the foothills (about 5,300 feet). Grassland with deciduous trees along watercourses only.

2. *Transition:* The foothill region up to about 8,000 feet: Characterized by an open growth of rock pine (*Pinus scopulorum*).

3. *Canadian:* Includes the intermediate mountain elevations up to about 10,500 feet. Lower portion characterized by a dense growth of lodge-pole pine (*Pinus murrayana*) which gives place at about 10,000 feet to a close forest of Engelmann spruce (*Picea engelmanni*) and balsam fir (*Abies lasiocarpa*).

4. *Hudsonian:* A rather narrow strip characterized by a dwarfing of the spruces and firs of the Canadian Zone and bounded above by timberline, a sharply defined but jagged division line occurring roughly at about 11,000 feet.

5. *Arctic-Alpine:* An area without trees or woody plants except some scrub willows. Huge snowdrifts last far into the summer or do not entirely melt. Summer temperatures are accordingly low and the season is short.

II. The Species of Entomostraca.

Table I. gives the list of species recorded for this area, and indicates for each the zones within which it has been collected. Of course it must be recognized that to make these data of final importance, very much more extensive collections are needed. It is apparent, however, that even the incomplete records here presented are sufficient to bring out facts of interest. The records for the more abundant species are sufficient to give wholly dependable results while the scattered records of the rarer ones are accurate as far as they go, but leave deficiencies and blanks to be filled by subsequent collections.

I have also included in Table I. the zones inhabited by each of these species throughout the Continent as a whole. These data are in many cases incomplete and in others uncertain because I have not had access to literature where I might find detailed ac-

TABLE I.

SHOWS ZONAL DISTRIBUTION AND GENERAL RANGE OF EACH SPECIES RECORDED FROM COLORADO.

Zones occupied in Colorado, as indicated by local collections, are designated in the upper line: LA, UA, T, etc. The probable distribution among the life zones throughout the continent of North America is indicated in the lower line: la, ua, t, etc. The Tropical Zone is omitted because of difficulty of determining with certainty which species enter it. A brief statement of general range is given in the last column. Records in parenthesis are for species not in my collections.

Species.	Number of Lakes	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
Branchinecta coloradensis Packard	7		UA				AA	Colorado.
Diaptomus shoshone Forbes	39				C	H	AA	Colo., Yellowstone.
D. arapahoensis Dodds	4						AA	Colo.
D. coloradensis Marsh	29			T	C	H	AA	Colo.
D. lintoni Forbes	2				C			Colo., Yellowstone.
D. judayi Marsh					(C)			Colo.
D. nudus Marsh	7			T	C	H		Colo.
D. leptopus var. *piscinae* Forbes	27			T	C	H		Colo., Mont., Alberta, Manitoba.
				t	c	h?		
Macrothrix montana Birge						(H)		Rockies and Sierras.
						h		
Limnetis gouldii Baird	2			T	C			Ill., New England, Quebec.
			ua	t	c			
Latona setifera (O. F. M.)	1				C			Northern U. S. and Europe (rare).
				t	c			
Holopedium gibberum Zaddach	11				C	H		Yellowstone, Sierras North U. S. and Europe, Newfoundland, Greenland, Iceland, Alps.
				t	c	h	aa	
Eurycerus lamellatus (O.F.M.)	10			T	C	H		Yellowstone, Sierras, North U. S. and Europe, Iceland.
				t	c	h	aa	
Acroperus harpae Baird	12			T	C	H		Yellowstone, Sierras, North U. S. and Europe.
				t	c	h		
Depanothrix dentata (Euren)	2				C			Me., Mich., Wis., Europe.
				t	c			
Camptocercus rectirostris Schoedler	2				C			Sierras, U. S., Europe.
				?	c	?		
Pleuroxus procurvatus Birge	5			T	C			Northern U. S.
				t	c			
Alonella exigua (Lilljeborg)	1				C			Me., Mich., Wis., Europe.
				t	c			

TABLE I.—*Continued.*

Species.	Number of Lakes.	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
A. excisa (Fischer).....	2			T				U. S., Europe,
			ua	t	c	h	?	Greenland.
Canthocamptus staphylinoides Pearse.....	4				C	H	AA	Nebraska.
			ua					
Streblocerus serricaudatus	1				C			La. to Wis., Nor-
(Fischer).....		la	ua	t	c	?		way, Sweden, Europe in general.
Macrothrix hirsuticornis	11		UA	T	C	H	AA	Maine to Colo.
N. & B.....		la	ua	t	c	h	aa	Spitzbergen to north Africa.
Alona affinis (Leydig)....	15		UA	T	C	H	AA	America and Eu-
		la	ua	t	c	h	aa	rope from north to south.
Alona guttata Sars.... .	4		UA	T	C			Wide spread in Am-
		?	ua	t	c	?		erica and Europe.
A. rectangula Sars.....	22			T	C	H	AA	Wide spread in Am-
			ua	t	c	h	aa	erica and Europe, Pribilof Ids.
Daphnia longispina	48			T	C	H		Cosmopolitan with
O.F.M.....		?	ua	t	c	h	?	many varieties.
Daphnia pulex DeGeer...	48		UA	T	C	H	AA	Cosmopolitan with
		la	ua	t	c	h	aa	many varieties.
D. hyalina Leydig..	1		UA	T	C			Wide spread in Old
		?	ua	t	c	h	?	and New Worlds.
D. psittacea Baird.... .	6		UA					U. S., Greenland to
		la	ua	t	c	h	aa	Algiers.
Ceriodaphnia reticulata	22		UA	T	C	H	AA	Cosmopolitan.
Jurine.....		la	ua	t	c	h	aa	
C. pulchella Sars.....					(C)			Cosmopolitan.
		la	ua	t	c	h	?	
Chydorus sphaericus	71		UA	T	T	H	AA	Cosmopolitan.
(O.F.M.)..... . ..		la	ua	t	c	h	aa	
Cyclops albidus Jurine....	16		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
		la	ua	t	c	h	aa	
C. bicuspidatus Claus....	38		UA	T	C	H	AA	Northern portions
			ua	t	c	h	aa	of America and Europe.
C. serrulatus Fischer.....	30		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
C. viridis Jurine..	45		UA	T	C	H	AA	Cosmopolitan.
		la	ua	t	c	h	aa	
Graptoleberis testudinaria	7		UA	T	C	H		U. S. and Europe.
(Fischer).....		la	ua	t	c	h	aa	Greenland.
Simocephalus serrulatus						(H)		Cosmopolitan.
(Koch).....		la	a	t	c	h	?	
S. vetulus (O.F.M.).....	26		UA	T	C	H		Cosmopolitan.
		la	ua	t	c	h	?	
Scapholeberis mucronata	14		UA	T	C			Yellowstone, Sier-
(O.F.M.).....		la	ua	t	c	h	aa	ras, Greenland. Arctic to tropics.

TABLE I.—*Continued.*

Species.	Number of Lakes	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
Bosmina longirostris (O.F.M.)	3	la	UA ua	t	c	h	?	Yellowstone, Sierras. America and Europe from north to south.
Canthocamptus minutis Claus	1	la	(UA) ua	t	C c	h	?	Wide spread in America and Europe.
Dunhevedia crassa King	3	la	ua	T t	C c			La. to New England and Wis. Europe, Australia.
Streptocephalus coloradensis Dodds	4		UA	T	T			Colorado.
Lepidurus bilobatus Packard				(T)				Colorado.
Estheria mexicana Claus		la	(UA) ua	t	c			Lake Winnipeg to Mexico; Ohio to Rocky Mts.
Diaptomus signicauda Lilljeborg				(T) t	c			Colo., Sierras.
D. siciles Forbes			(UA) ua	t	c			Neb., Wis., Yellowstone, Muskoka Lakes, Great Lakes.
Pleuroxus adunctus Jurine	1	la	UA ua	t	c	?		Colo., Calif. Northern Europe to Algiers.
P. denticulatus Birge	3	la	UA ua	t				All parts of U. S.
Leydigea quadrangularis (Leydig)	1	?	UA ua	?				U. S. and Europe.
Kurzia latissima (Kurz)		?	(UA) ua	?				U. S. and Europe.
Moina brachiata (Jurine)	7	la	UA ua	t				Wis. and southward. Europe to Egypt.
Cyclops ater Herrick		la	(UA) ua	t				Minn. and Mich. to Alabama.
M. affinis Birge		la	(UA) ua	t				Wis. to La.
Diaptomus pallidus Herrick	1	la	UA ua	t				Wis. to La.
D. siciloides Lilljeborg	2	la	UA ua	t				Wis. to Texas and Calif. (L. Tulare).
D. claviceps Schacht	2		UA ua					Colo., Iowa, Neb.
D. albuquerquensis Herrick	1	la	UA ua					Colo., N. M., Mexico City.
Marshia albuquerquensis Herrick	1	?	UA ?					Colo. and (N. Mex. ?)
Apus lucasanus Packard	2	la	UA ua					Colo., Kan., Lower Calif.
A. aequalis Packard	1	la	UA ua					Colo., Kan., Texas, Mexico, Lower Calif.

TABLE I.—*Concluded.*

Species.	Number of Lakes	Zones.						Range.
		Lower Austral.	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.	
A. mewberryi Packard..			(UA)					Colo., Utah.
A. longicaudatus Leconte			(UA)					Colo., Neb., Texas,
		?	ua	?				Montana.
Eulimnidia texana			(UA)					Colo., Neb., Kan.,
Packard..... ..		?	ua					Texas.
Estheria morsei Packard	2		UA					S. Dak. to Colo.
			ua					
E. compleximanus Pack-			UA					Colo., Kan., Lower
ard	1	?	ua					Calif.
Streptocephalus texanus	2		UA					Colo., Neb. to
Packard.....		?	ua					Texas.
Thamnocephalus platyu-	1		UA					Colo., Kansas.
rus Packard.....			ua					
Branchinecta packardi	2		UA					Colo.
Pearse.....								
B. lindahli Packard....			UA					Colo., Kan., Neb.,
			ua	t?				Wyoming.

counts of localities where each species has been collected. Many of the records are indefinite or very general and do not enable one to judge just what zone is included. The tabulation, in spite of this, contains much of truth and I have attempted to confine its errors to those of omission. The Tropical Zone has been omitted, chiefly because I have been unable to determine certainly which species in my list extend into it. It is likewise difficult to determine which species range into the Arctic-Alpine.

The agreement between the local and general zonal range is striking, and in many cases, complete. The only departures from this agreement are in the cases of certain species for which the local records do not cover as many zones as the general. These species are mostly those which are not abundant and have been met with infrequently in the Colorado collections, such as certain species which are never present in more than very small numbers and may easily be missed in making collections, or others, which though found occasionally in abundance, are met with in only a small proportion of the lakes in the zones which they inhabit. More extensive collections would doubtless extend the records of many of these species to cover other zones and so bring the local records into still closer agreement with the general.

III. The Fauna of Each Zone.

Table II., summarizing the fauna of the different zones, shows the following points of interest. For the species collected in Colorado there is a decrease in number from the Upper Sonoran upward, that is, a thinning out of population as the more extreme conditions of climate are met. An apparent exception is the Transition Zone where the number is less than in the Canadian. This is no doubt due to the fact that in the region assigned to this zone there are very few lakes and the number of collections is accordingly much less than from any other zone. If we add to the number of species actually collected from each zone in Colorado those which from their general distribution are almost certainly to be expected there, the deficiency of the Transition Zone is made up and the general decrease with each successive zone prevails.

Table II.

Summary of Records Given in Table I.

	Upper Austral.	Transition.	Canadian.	Hudsonian.	Arctic-Alpine.
Number of species collected in each zone in Colorado.....	44	28	35	23	15
Number of species in the Colorado list to be expected in each zone as determined by comparing local and general records .	52	50	42	34	28
Total number of species collected in Colorado in all zones....	71				

The Upper Sonoran Zone.

In the Upper Sonoran Zone of this region there occur two kinds of ponds or small lakes: transient pools which follow rains, and ponds and reservoirs filled with water from irrigating ditches. These latter are of recent origin, dating back only to the introduction of agriculture into this region. The fact that they are richly populated speaks well for the effectiveness of the methods of dispersal of plankton Crustacea, but the question may well be raised whether dispersal has kept pace with the increase in number of such bodies of water and whether additions to their fauna may not be expected in future years.

Of the 44 species that have been recorded from this zone in Colorado, 28 have not been found in any of the higher zones,

though nine or more of them occur in the Transition zone of our northern states and may be expected to extend into the local portion of this zone. This leaves 17 species which apparently do not go above the Upper Sonoran, to which must be added some others which have their greatest abundance here rather than in the Transition into which they extend. The remaining species of the zone, roughly half of its population, range upward into other zones, 9 of them as far as the Arctic-Alpine. Thus we recognize among the species of this zone two groups: the one including euthermic species which range upward across temperature lines into the higher zones, the other composed of stenothermic forms, intolerant of great temperature differences and accordingly confined pretty strictly to the zone. The euthermic species, while they form at least half the fauna of the zone, are less likely to receive consideration on account of zonal distribution because they do not form its distinctive part. To this group belong nearly all the species of the family Daphnidæ, many of the Chydoridæ and four of the five species of *Cyclops,* together with a few from other groups.

Conspicuous in the stenothermic component of this zone are two groups: (1) four species of *Diaptomus* and (2) eleven species of phyllopods (Anostraca, Notostraca, and Conchostraca). These two groups are of interest because, not only in this region, but in general they are represented by stenothermic species. *Diaptomus* forms an important part of the entomostracan fauna everywhere and includes many species, nearly all of which are very narrowly stenothermic and have restricted geographic ranges. The phyllopod group is of special interest here, for, though represented in the fauna of all parts of the country, and of the world, forms a more important part here than in most regions. The species of this group are especially abundant in the Sonoran portion of the Austral Zone, where they flourish in the transient pools of this arid region. It will be seen by reference to the last column of Table I., that these species are all of restricted range and confined to the states which include portions of the Sonoran zone. The species of phyllopods, like those of *Diaptomus,* are nearly all stenothermic. The phyllopods belong in greater abundance to the warmer zones, while *Diaptomus* is especially plentiful in the colder ones.

When we search for distinctive faunal characters of the higher zones we are confronted with a problem somewhat different than in the Sonoran. In no other zone is there even a single species confined to it and at the same time abundant enough to be used as a zone indicator. In certain respects the zones of the mountain region, Transition to Arctic-Alpine, form a unit group, which, as a whole, may be contrasted with the Sonoran. There are 23 species which have been collected exclusively in the mountains, many of them in considerable abundance, which clearly belong to the colder zones. Yet when we attempt to apportion them among the zones it is found that most of them range through two or more zones. There are, however, certain pretty definite faunal characters of each zone, a description of which follows.

The Transition Zone.

In the foothill region of the mountains there are but few lakes or ponds and accordingly few collections have been made and a small number of species recorded from this zone. This leaves some doubt as to the nature of the transition between the fauna of the plains and that of the mountains. We cannot determine whether this is truly a transition zone or if it is one having special and striking characters of its own, though the former view seems more probable. It is further probable that the fauna of this zone partakes more of the nature of the higher than of the lower zones. While there are 23 species which make the Transition Zone their lower limit, there is not one which finds its upper limit in this zone, though from the records of general range, there are some which may be expected to do so when more extensive collections have been made here. Twenty-eight species have been recorded in this zone, but a total of 50 might be expected because certain others of the Colorado list are present in the Transition of our northern states.

The Canadian Zone.

In the belt assigned to this zone there are many small lakes, nearly all of morainal origin, mostly shallow, often without outlet, and commonly surrounded by forest. Their fauna is rich in individuals and species. There have been collected in the lakes of this zone 37 species, and about 8 others might be expected. This

zone is not one of importance as a limit in either direction, for no such significance can be assigned to the five species from the Upper Sonoran which make this their upper limit or to the three which find their lower limit here. Its fauna does, however, include a number of species in considerable abundance which are common forms in the continental Canadian and other northern zones. Important among these are *Limnetis gouldii, Latona setifera, Holopedium gibberum, Eurycerus lamellatus, Acroperus harpæ,* and *Pleuroxus procurvatus.* Six species of *Diaptomus* common in this zone, *D. shoshone, D. coloradensis, D. lintoni, D. nudus, D. judayi,* and *D. leptopus* var. *piscinæ* are confined more or less closely and locally to the higher parts of the Rocky Mountains, where they range through more than one zone.

Of special interest in the Canadian Zone of this region is the great abundance and very frequent association together of *Daphnia longispina* (subject to great local variation) and *Diaptomus leptopus* var. *piscinæ,* which throughout this zone form the dominant species, with *Diaptomus coloradensis* as the form of next importance. *D. longispina,* though a euthermic species and found throughout the world in many varieties, has not been found in Colorado below the Transition and has by far its greatest abundance in the Canadian. *D. leptopus* var. *piscinæ* seems to be confined to the Transition, Canadian, and Hudsonian of the Rocky Mountain region, while *D. coloradensis* is a local species and has not been reported outside the mountain region of the state for which it is named. The list of euthermic species is about the same as in the Upper Sonoran.

The Hudsonian Zone.

This is a narrow zone, both in vertical and in geographic extent, and has only slight significance. Climatic conditions are more severe than in the Canadian; more snow falls and it lies longer; the summer is shorter and the lakes are colder. In these respects it approaches the Arctic-Alpine. Its lakes are of two sorts: the one of the same type as those common in the Canadian, the other more like those of the Arctic-Alpine—lakes on the direct courses of the creeks like those higher up but differing from them in that they are surrounded by timber and have considerable silt on

their bottoms. Lakes of the former type in this zone have a fauna of much the same nature as in the Canadian, while those of the latter kind have their affinities, though less decidedly so, with the Arctic-Alpine. A few species make this zone their upper limit and it seems probable that it may have some importance in this respect. In the main, it seems to be a transition zone, one having no important distinctive characters and it might well be included within the Canadian.

The Arctic-Alpine Zone.

This zone is an interesting one partly because of a type not familiar to us, and partly because its fauna is distinctive and striking. The lakes of this zone lie in the deep cirques at the heads of the streams and on the stream courses not far below, and are all just at or above timberline. Most of them are deep and clean and are bordered by steep, rocky slopes. Ice remains till June or July or even later, and the snowdrifts on the surrounding walls furnish cold water throughout the greater part of the summer so that the highest temperatures reached range from 45° to 55° F. Shallow pools of this region have about the same type of fauna and must clearly be classed along with the lakes of this zone.

Fifteen species have been collected here, nine of which are widely euthermic and range through all the zones, not only to the Upper Sonoran of Colorado, but into the Lower Sonoran and Austral, and in some cases into the Tropical. Nearly all of the more abundant species of the zone belong to this group, as *Daphnia pulex, Chydorus sphaericus, Alona rectangula,* and four species of *Cyclops.* Species of more restricted zonal range are, however, important and abundant, though none of great importance is confined to the zone. Most of the northern species listed as of importance in the Canadian fail to reach this zone, but find their highest range in the Hudsonian, though it appears from records of their presence in Greenland and other high northern regions that they might be expected here. An abundance of collections in this region has not, however, found them in the Arctic-Alpine of this region, and we must omit them from its list.

The two most conspicuous and abundant species are *Diaptomus*

shoshone (33 lakes out of 43) and *Daphnia pulex* (27 lakes). One or both of these species were found in 39 of 43 lakes in this zone. *Diaptomus shoshone* is a large and brilliantly colored species, was described by Forbes from the Yellowstone, and has been reported only from that region and high portions of the Rockies in Colorado. It ranges into lakes far within the Canadian Zone, but has by far its greatest abundance in the lakes and pools above timberline. *Daphnia pulex* is a cosmopolitan and euthermic form but is much more abundant in these high lakes than in any other part of the mountains, in fact is nearly wanting from the Hudsonian and Canadian, where it is replaced by *Daphnia longispina* just as *Diaptomus shoshone* is replaced by *D. leptopus* var. *piscinæ*. The variety of *Daphnia pulex* found in these lakes is unusually large, and though subject to considerable local variation, comes close to the form described from the Yellowstone as *D. clathatra* by Forbes, where, too, it is associated with *D. shoshone*. One of the striking and outstanding features about zonal distribution in this region is this definite and constant association of a species of *Daphnia* with one of *Diaptomus*. The two combinations are striking and constant, one belongs definitely to the Canadian, the other to the Arctic-Alpine, and the reverse combination is seldom met. Neither member of the Canadian pair has been collected from lakes in the Arctic-Alpine, and though both members of the alpine pair do sometimes invade the Canadian, they usually go together and are found in lakes where the other pair is wanting. *Diaptomus coloradensis* is frequently found in the Arctic-Alpine but is of secondary importance just as in the Canadian.

Another species of importance in this zone is the fairy shrimp, *Branchinecta coloradensis* which is commonly found in the pools, though never in the lakes, along with *Daphnia pulex* and *Diaptomus shoshone*. This species was described by Packard from an elevation of 12,000 feet on Gray's Peak, has been repeatedly found in these high pools, and has come to stand as a type of a distinctly alpine species of narrow range (Shantz, '05). Only once have I collected it below the Arctic-Alpine, this at 9,575 feet, well within the Canadian Zone, and there is was associated with the same species of *Daphnia* and *Diaptomus* as in the higher

regions. I have one record, however, from a collection dated May 30, 1912, sent me by Professor Max M. Ellis from St. Vrain, Colorado, at an elevation of about 5,100 feet and well within the Upper Sonoran. It seems improbable that there can be any mistake about the label of this collection, and I have repeatedly looked at the material in an attempt to detect differences which would relieve me of the necessity of referring it to this species. This record places it at once in the Upper Sonoran and calls for a revision of our notions of its distribution.

IV. Summary and Discussion.

The zonal distribution, local and general, of the 71 species included in this report affords good material for the study of certain characteristics of euthermic and stenothermic species belonging to closely related families and genera. It is at once apparent that the total entomostracan population of this region is readily divisible into these two components. The euthermic species not only range through several zones in their local and general distribution, but a large proportion of them are essentially cosmopolitan, being reported from all parts of the world where collections have been made. The stenothermic species are divisible into two groups, one belonging to the colder, the other to the warmer zones. The stenothermic species of the colder zones may in turn be resolved into two groups, one of which includes species that range over a considerable area from Colorado northward into the corresponding zones of our northern states, Canada, and even into Greenland, Iceland, and northern Europe, while the other includes species with very narrow ranges, confined to the higher parts of the Rocky mountains. The stenothermic species of the warmer lakes (the Upper Sonoran of Colorado) may likewise be divided into two groups, one, of species ranging over the Sonoran, and through the Austral in general, and into the Tropical Zone, the other, of those with a narrow range in the Upper Sonoran of a few states.

It is further evident from a scrutiny of the facts presented in Table I., where the species are arranged on the basis of temperature toleration, that such a grouping bears a certain relation to taxonomic groups, inasmuch as some of these groups are

largely stenothermic and others euthermic. The outstanding features as seen in the Colorado list are as follows:

The 16 species of phyllopods in the list are, with two exceptions, narrowly stenothermic and all but one of the stenothermic species belong to the warm-climate fauna. It is moreover true that nearly all of the known species of the group are stenothermic and have narrow geographic ranges. Among the Cladocera, the Family Daphnidæ is represented by 11 species which are nearly all euthermic in a broad sense, are found at all elevations and in all zones, and are decidedly cosmopolitan, while the Chydoridæ has 16 species about equally divided between stenothermic and euthermic types. The Copepods include two sharply contrasted genera, *Cyclops* and *Diaptomus*, both of which form important components of the entomostracan fauna in all parts of the world. *Diaptomus* has 13 species in the Colorado list, not one of which is euthermic in any broad sense, and all of which are confined within areas of very limited extent. *Cyclops*, on the other hand, with only 5 species, is fully as important. Three of these are broadly cosmopolitan and euthermic, a fourth (*C. bicuspidatus*) is nearly so, being absent only from the warmer waters, and the fifth (*C. ater*), confined to the United States and not found north of the Transition Zone, has as great a range as the widest spread species of *Diaptomus*.

I believe the collections of Entomostraca described in this paper are the most extensive that have been made in this country from a region where high mountains cause such a decided narrowing of life zones. So far as I know, this is also the first attempt to define the entomostracan fauna of the various zones of such an area and to definitely place them with reference to Merrian's Life Zones. The data from Colorado lend themselves well to such an anaysis. The zonation is definite, even though the present collections do not enable us to discriminate between all of the colder zones. It is equally difficult, on the basis of our present knowledge, to sharply differentiate these same zones in their continental extent in Canada and northern United States.

Yet it seems well established by these data that the entomostracan population of the various life zones as they occur in the mountains of Colorado are but attenuated southern extensions

and isolated fragments of the broader life zones, from Transition to Arctic-Alpine, which extend from east to west across the continent. Though these zones have been established and defined on the basis of organisms other than entomostraca, yet the agreement is striking and real and extends even to common species inhabiting the same zones in localities hundreds of miles apart and separated by many degrees of latitude.

In this fact we are brought again to a recognition of the truth that change in elevation may, within a very few miles, produce climatic and biotic differences which are brought about by latitude, only after hundreds of miles. It shows clearly that life zones are neither latitudinal, nor altitudinal, though these are the two large factors which interact to produce them. We have not discovered an exact mathematical formula for determining life zones on the basis of these two factors, nor are we able to accurately define them on the basis of climatic conditions, but they may be defined and compared in terms of distribution of animals and plants. These, of course, serve as a measure of climatic conditions, that is, as these conditions determine the distribution of the several species. It is apparently not the case that temperature acts in exactly the same way on all species of animals and plants in determining their distribution. For some, it is apparently winter conditions which limit their range into colder regions, though for the great mass, it is probably summer conditions. It is not the purpose of this paper to enter into a discussion of this point, though the fact of winter-killing of certain species is well known, while it is recognized also that every species requires a certain minimum heat budget during the summer, extending over a longer or shorter period, in order that it may reproduce.

It is not to be expected that limiting lines for all species will be exactly parallel, yet there is a large agreement, so that the zones do, in a high degree, maintain their several identities in whatever proportions altitude and latitude interact in producing them. It would be of interest to investigate further into the extent and nature of this identity between the colder zones of the elevated parts of Colorado and the less elevated portions of these zones in the far north. The present study goes merely far enough

to demonstrate an agreement of a general nature without showing in detail its extent.

BIBLIOGRAPHY.

American Ornithologists' Union.

'10 Check-List of North American Birds. 3d ed. (revised). New York.

Beardsley, A. E.

'02 Notes on Colorado Entomostraca. Trans. Am. Micros. Soc., Vol. 23, pp. 40–48.

Cary, Merritt.

'11 A Biological Survey of Colorado. U. S. Dept. Agr., Bureau Biol. Surv., No. 33, 256 pp.

Cockerell, T. D. A.

'12 The Fauna of Boulder County, Colorado. II. Univ. Colo. Studies, Vol. 9, pp 41–52.

Dodds, G. S.

'08 A List of the Entomostraca of Colorado. Univ. Colo. Studies, Vol. 4, pp. 243–250.

'15a Description of Two New Species of Entomostraca from Colorado, with Notes on Other Species. Proc. U. S. Nat. Mus., Vol. 49, pp. 97–102.

'15b A Key to the Entomostraca of Colorado. Univ. Colo. Studies, Vol. 11, pp. 265–299.

'17 Altitudinal Distribution of Entomostraca in Colorado. Proc. U. S. Nat. Mus., Vol. 54, pp. 58–87.

Ekman, Sven.

'05 Die Phyllopoden, Cladoceren und Frilebenden Copepoden der nordschwedischen Hochgebirge. Zool. Jahrb. Abth. System, Vol 21, pp. 1–170.

Forbes, S. A.

'93 A Preliminary Report on the Aquatic Invertebrate Fauna of the Yellowstone National Park, Wyoming, and of the Flathead Region of Montana. Bull. U. S. Fish. Com., Vol. 11, pp. 207–258.

Juday, Chancey.

'07a A Study of Twin Lakes, Colorado, with especial Consideration of the Food of the Trouts. Bull. Bureau of Fisheries, Vol 26, pp. 147–178.

'07b Studies on Some Lakes in the Rocky and Sierra Nevada Mountains. Trans. Wis. Acad., Vol. 15, pp. 781–793.

Lilljeborg, L.

'00 Cladocera Suecia. Upsala, 701 pp. 87 pls.

Marsh, C. Dwight.

'07 A Revision of the North American Species of *Diaptomus*. Trans. Wis. Acad., Vol. 15, pt. 2, pp. 381–516.

'10 A Revision of the North American Species of *Cyclops*. Trans. Wis. Acad., Vol. 16, pt. 3, pp. 1067–1135.

Merriam, C. Hart.

'98 Life Zones and Crop Zones of the United States. U. S. Dept. of Agric., Division Biol. Survey. Bull. No. 10, 79 pp.

Packard, A. S.

'83 A Monograph on the Phyllopod Crustacea of North America, with Remarks on the Order Phyllocarida. U. S. Geol. Survey (Hayden), 12th Ann. Rept., Vol. 1, pp. 295–592.

Pearse, A. S.

'05 Contributions to the Copepod Fauna of Nebraska and Other States. Studies from Zoöl. Lab. Univ. of Neb., No. 65, pp. 145–160.

'12 Notes on Phyllopod Crustacea. Mich. Acad. Sci., 14th Report.

Ramaley, Francis.

'07 Plant Zones in the Rocky Mountains of Colorado. Science, N. S., Vol. 26, pp. 642, 43.

Shantz, H. L.

'05 Notes on the North American Species of *Branchinecta* and their Habits. Biol. Bull., Vol. 9, pp. 249–264.

Ward, Henry B.

'04 A Biological Reconnaissance of Some Elevated Lakes in the Sierras and Rockies. Report on the Cladocera by E. A. Birge. Report on the Copepoda by C. Dwight Marsh. Studies from Zoöl. Lab. Univ. Neb., No. 60.

Ward, Henry B., and Whipple, George C.

'18 Fresh-Water Biology. The Cladocera by E. A. Birge. The Copepoda by C. Dwight Marsh. The Phyllopoda by A. S. Pearse. John Wiley and Sons.

Wesenberg-Lund, C.

'08 Plankton Investigations of the Danish Lakes. General Part: The Baltic Fresh-water Plankton, its Origin and Distribution. 391 pp., 46 tables.

Zschokke, F.

'00 Die Tierwelt der Hochgebirgseen. Neu. Denkschr. allg. Schweiz, Ges. ges. Nat., 400 pp.

NOTES ON SOME PROBLEMS OF ADAPTATION.

I. On the Re-formation of the Mantle-glands of Chromodoris.[1]

W. J. CROZIER,

Hull Zoölogical Laboratory, University of Chicago.

I.

Upon the ventral surface of the mantle-fold which encircles the body of nudibranchs of the genus *Chromodoris* there is often found, at the posterior end of the fold, a set of small gland-like nodules producing conical elevations of the skin (Fig. 1). With some species these organs may be absent; in others they are relatively invisible during life, becoming more conspicuous, however, at the death of the animal. In *Chromodoris zebra* the glands during life are usually quite obvious, appearing as a number of white conical bodies rather evenly spaced about the margin of the caudal veil. Here, also, they become more prominent at death, due to increased fluid pressure in the tissue surrounding each gland. A similar degree of enhanced prominence, an enlargement of each conical eminence, is induced by intense stimulation of the nudibranch's skin. The glands apparently act as reservoirs of a repugnatorial integumentary secretion. When the animal is strongly stimulated, the secretion may be seen to flow from the terminal pores of one or more of the conical elevations (Crozier, '16). I refer to these bodies as "glands," loosely; the extent of their truly glandular activity is not yet decided.

The number of glands present on the caudal veil of an individual varies considerably, namely from 0 to 19. In connection with an analysis of the ethology of *C. zebra,* I found it necessary to investigate the physiology of these glands, and to study their numerical variations. From this study certain inferences may be derived as to the nature of the processes leading to the

[1] Contribution from the Bermuda Biological Station for Research. No. 120.

development of the glands. If these inferences be correct, an interesting parallel is afforded with a law of regenerative phenomena in plants (cf. Loeb, '15; '18*a*; '18*b*).

II.

The outline of the caudal veil is characteristically smooth and approximately semicircular. In a certain number of cases, however, there are apparent in this outline obvious irregularities of a secondary nature. Among the individuals presenting a regular,

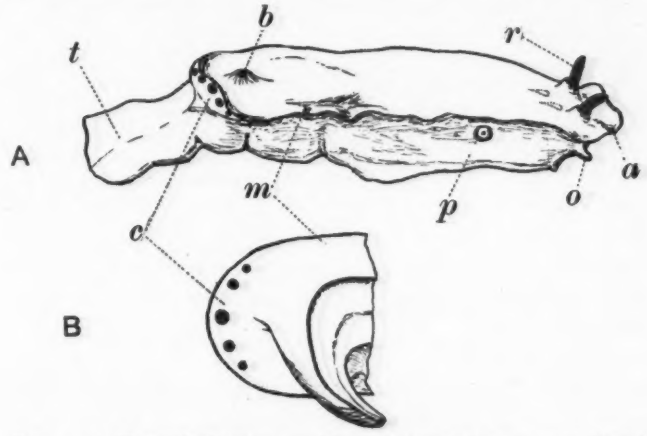

FIG. 1. *A. Chromodoris zebra*, caudal veil (*c*) turned up so as to show the glands (black dots) on its ventral surface; *a*, buccal veil; *b*, branchial collar, contracted over the concealed branchiæ; *m*, mantle folds; *o*, oral tentacle; *p*, reproductive papilla; *r*, "rhinophore"; *t*, "tail" of the foot (× ¾).

B. The caudal veil in ventral aspect, "tail" part of foot turned to one side; this shows a plan of distribution that, on the whole, is the most common.

unindented margin, the number of glands varies from 1 to 16 (Fig. 2). The most common arrangement of the glands is, that one is median, with two on either side of it, all evenly spaced (Fig. 1, *B*). Typically, the five glands in such a set are of about the same size, although in many cases the median gland is the largest.

Although five is the modal number of glands (Fig. 2), other numbers are common; some of these arrangements are illustrated in Fig. 3. Not infrequently one or another gland in an other-

wise symmetrical distribution seems omitted, or suppressed (Fig. 4). It seems possible, if not probable, that each gland may undergo a more or less cyclic sequence of growth and shrinkage.

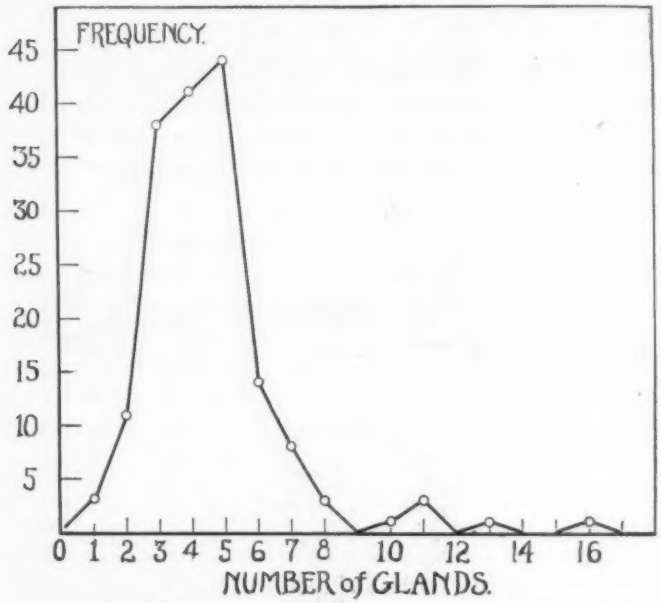

FIG. 2. Showing the frequency with which different numbers of glands occurred in a series of 166 individuals; no obviously irregular sets of glands were included (see text).

Independently of such variations, there is a still further diversity of numbers and sizes among the different sets. As to the number of glands, some examples are given in Fig. 3. In the instances illustrated, and indeed throughout series of several hun-

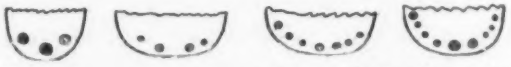

FIG. 3. Examples of symmetrical sets of "glands" (× 2/3).

dred animals each, examined at different times since 1915, it is generally true that if the number of glands in a set is low, their size is individually larger than in the case of groups composed of greater numbers. Within certain limits, which do not concern

the series of individuals here involved, this is true without reference to the sizes of the nudibranchs. As shown by Fig. 5, the number of glands present in symmetrical sets is independent of the size of the animal.

This leads to the idea that, in general, only a definitely limited

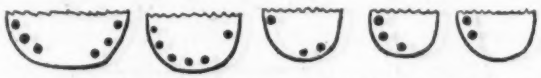

FIG. 4. Examples in which one or more "glands" are omitted (× 2/3).

amount of material is available in each animal for the formation of the white mantle-bodies, regardless of the *number* of these bodies which may be present.

LENGTH, CMS.

NUMBER OF GLANDS.	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0							1							
1						1	1							
2					1	1	3	2	4	1				
3			2	2	8	6	6	7	7					
4	1		1	1	4	8	11	6	7	2	3			
5		2	1	5	13	13	11	6	5	2	3		2	2
6	1				1	7	5	5	2		2			
7				2	1	2	3	2						
8							1		2					
9														
10				1		1								
11								1						
12														
13							1							
14														
15														
16							1							

FIG. 5. Showing the absence of sensible correlation between the *number* of glands on the caudal veil of *Chromodoris* and the *size* of the nudibranch (203 individuals), among instances exhibiting a normal symmetrical distribution of the glands. The column of modal length and the row of modal frequency of glands are enclosed between parallel lines.

III.

A frequency-distribution of the numbers of glands found on different individuals has been given in Fig. 2. In order to avoid certain non-pertinent complications, perhaps resulting in part from seasonal differences (Crozier, '19*a*), I have included in

Fig. 2 only data from one series, obtained September 21–30, 1918. In addition to the individuals involved in Fig. 2, 17 *Chromodoris,* or 9.3 per cent. of the total collected at this time, were

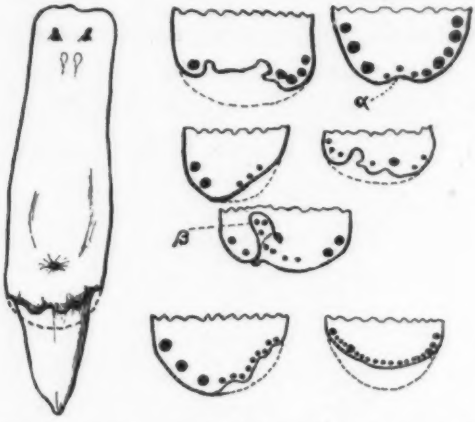

Fig. 6. Showing the character of injuries suffered by the caudal veil, and the results of removal of glands; at α, probably one gland had been removed; at β, a tongue of torn tissue; original outlines approximately as indicated in dashed lines (× 2/3). See text.

seen to have the margin of the caudal veil markedly irregular. Instances of this sort are shown in Fig. 6.

It has nearly always been true in cases of marked irregularity of the caudal mantle that several small glands are situated at

Fig. 7. In *a*, three small glands (surrounding the location of a shrunken gland?); at *b*, a condition which may result from the subsequent enlargement of a gland much shrunken for a time; at *c*, the inclusion (?) of a small gland by an enlarging one normally situated (× 2/3). See text.

points where but one such organ, and that a large one, would characteristically occur. This has been interpreted to mean that if, as the result of injury, one or more glands have been removed, material constantly being transported to these regions is then collected into a greater number of small glands.

It is in fact known that injuries are suffered by the mantle-fold and by other parts of *Chromodoris,* mainly (if not entirely) through the biting of fishes (cf. Crozier, '19*a*). The re-formation of the caudal veil, and with it of mantle-glands, after amputation, has been observed in the laboratory. The process was slow, however (Crozier, '16), occupying some 4 months, and was therefore unsuitable for experimental study; the tissue of the mantle-fold is regenerated very slowly indeed, new glands appearing before an excised region of the caudal veil is restored.

The view here advanced involves the idea that the major portion of the gland-contents, or the precursors of these contents, are in some way carried from distant parts of the animal to the site of the glands. It can be pointed out that the repugnatorial substances contained in the glands are indeed present in the general mantle surface of the nudibranch. Several instances were noted wherein a nudibranch with a much injured caudal veil exhibited 3 or 4 small white accumulations of secretion on the ventral surface of the *buccal* veil. A detailed account of these secretions, with proof of their repugnatorial character and function, I shall provide in another publication.

Microscopically, each gland consists of a spherical sac, tightly packed with oily globules of a special kind, and communicating with the outside by means of a minute pore. In *C. zebra* the histological conditions are not unlike those earlier figured (somewhat incompletely) by Bergh ('98) for *C. juvenca.* Bergh did not observe the pore-like opening, which is difficult to see in sectioned material, and indeed is absent save in mature glands, and at this time the real nature of these structures was unknown. On some other portions of the mantle fold, notably on the ventral surface of the buccal veil, minute white bodies are occasionally seen, which give rise to a similar secretion; these bodies are never so large as the caudal glands.

It has been remarked that each gland may perhaps undergo a process of shrinkage, followed by growth to a maximum size. This might explain such cases as are illustrated in Fig. 7. Materials ordinarily going to a particular gland might, if this gland be in a phase of shrinkage, accumulate in several smaller, new glands about its periphery; subsequent re-growth of the original

gland could then result in a condition such as is shown in Fig. 7, the further development of the new glands being prevented.[1]

IV.

Loeb ('15, '18*a*, '18*b*) has sought to account for certain fundamental characteristics of organ-formation, as seen in *Bryophyllum*, on the basis of a flow of "organ forming" materials. In the case of buds arising from notches in an isolated leaf of this plant, the buds first growing out attract to themselves materials available in the leaf for the growth of buds. The total amount of such materials being limited, the growth of these first buds automatically inhibits the development of others. This explanation has the advantageous support of really quantitative experiments, and it is of interest to notice how closely it may apply to the not very dissimilar phenomena of gland re-formation on the caudal veil of *Chromodoris*.[2]

Regeneration experiments have made it probable that the supply of materials to the region of the glands is so slow that over any given period the total available quantity of such material may be regarded as fixed. The relative sizes of the several glands in a set, in cases where size differences are evident, show that a certain proportion of the gland materials is normally received by each of the (commonly 5) regions of the caudal veil where a gland is characteristically developed. So long as the original glands are present in full activity, no further formation of glands seems to take place. If, however, some of these be removed, the current of secretory materials continues to this region of the mantle and results in the formation of an excessive number of new glands, which individually remain of relatively small size.

[1] A distinct tendency is noticeable for the more anterior glands of symmetrical sets to be smaller than the medial ones, pointing to a bilateral scheme for the distribution of the pro-secretory materials. The exact conditions attending the development of a gland at a particular place can be made clear only from histological study. It seems probable that any portion of the epithelium of the caudal vein *can* give rise to a gland.

[2] Some perhaps analogous phenomena have been described in the growth of previously injured Madreporarians, where normally there is a "dominant apical zooid" (cf. Wood-Jones, '12, p. 112). The manner in which exhausted gland-cells of the amphibian skin are replaced by the enlargement of previously "dormant" cells is also suggestively similar.

The size relations make it clear that what might loosely be regarded as an adaptive over-production of repugnatorial glands subsequent to injury, probably results, on the contrary, from (1) the limited quantity of the repugnatorial precursor substances, and (2) the fact that normally the development of new glands is inhibited through the attraction ("drainage") of these substances by glands already established. This will also explain why in cases where, for example, but 3, rather than 5, "glands" are present on an undamaged mantle, the "glands" are in the former instance individually larger. What it is that determines the number of glands originally formed, remains undiscovered; they are probably absent in all individuals of *C. zebra* less than 1 cm. long.

CITATIONS.

Bergh, R.

'98 Die Opisthobranchier der Sammlung Plate. Zool. Jahrb., Suppl.-Bd. (Fauna Chilensis, 1^te Bd.), pp. 481–582.

Crozier, W. J.

'16 On the Immunity Coloration of Some Nudibranchs. Proc. Nat. Acad. Sci., Vol. 2, pp. 672–675.

'17 The Nature of the Conical Bodies on the Mantle of Certain Nudibranchs. Nautilus, Vol. 30, pp. 103–106.

'19a Further Contributions upon the Natural History of *Chromodoris zebra*: the Question of Adaptive Coloration. Anat. Rec., Vol. 15, p. 349.

'19b On the Control of the Response to Shading in the Branchiæ of *Chromodoris*. Jour. Gen. Physiol., Vol. I., pp. 585–591.

Loeb, J.

'15 Rules and Mechanisms of Inhibition and Correlation in the Regeneration of *Bryophyllum calycinum*. Bot. Gaz., Vol. 60, pp. 249–276.

'18a Chemical Basis of Correlation, I. Ibid., Vol. 65, pp. 150–174.

'18b The Law Controlling the Quantity of Regeneration in the Stem of *Bryophyllum calycinum*. Jour. Gen. Physiol., Vol. 1, pp. 81–96.

Wood-Jones, F.

'12 Coral and Atolls. xxiii + 392 pp., London [Reissue].

NOTES ON SOME PROBLEMS OF ADAPTATION.

2. On the Temporal Relations of Asexual Propagation and Gametic Reproduction in Coscinasterias tenuispina: with a Note on the Direction of Progression and on the Significance of the Madrepores.[1]

W. J. CROZIER,

Hull Zoölogical Laboratory, University of Chicago.

I.

Some time ago I gathered evidence showing that in the common Bermuda starfish, *Coscinasterias tenuispina* (Lamk.), there is unmistakable evidence of asexual multiplication through spontaneous division of the body into two parts (Crozier, '15). The modal number of rays in the adults of this species is 7[2]; the parts resulting from self division commonly constitute either 3 or 4 rays; each of these parts most usually produces 4 new rays. I may give here some additional data obtained in subsequent years, leading to the same conclusion (Fig. 1, Table I.).

Table I.

The Relation between the Number of Long and the Number of Short Rays in 237 Coscinasterias, of which Each Individual had Two Distinct Groups of Rays.

The table shows that as a rule 4 short rays are found in association with either 3 or 4 long rays.

SHORT RAYS.	LONG RAYS: 1	2	3	4	5	6	7	
1			1	1	5	5	3	15
2			6	5	8			19
3		2	18	32	5			57
4		8	42	55	4			109
5		7	17	7	1			32
6	1	3			1			5
	1	20	84	100	24	5	3	237

[1] Contributions from the Bermuda Biological Station for Research. No. 121.

[2] It is entirely probable that here, as in *Solaster* (Gemmill, '12) and in *Leptasterias* (Osterud, '18), the fundamental ray number is 5, to which at an early age a sixth and, later, a seventh ray are normally added (cf. Crozier, '15).

The ordinary, gametic reproduction of these starfishes takes place in January–February. During summer months they are quite abundant under stones along the shore, just under low-water level. As winter approaches, they retreat for the most

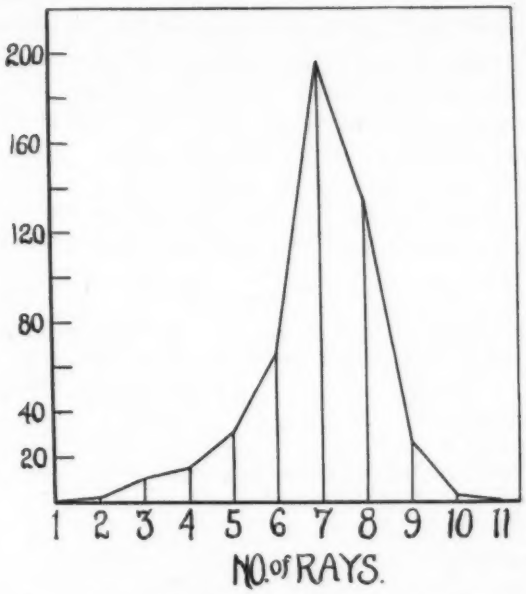

FIG. 1. The ray-frequency polygon derived from counts of 487 *Coscinasterias*. The essential characteristics of this curve are identical with those of a curve previously published (Crozier, '15).

part to somewhat deeper water, it would appear. In January and February, however, they crowd up to high-water mark, even on exposed shores, within Great Sound at least, and in Harrington Sound.[3] Often they are consequently left high above water level

[3] The question presents itself as to the possible rôle of phototropism in such shoreward movements. *C. tenuispina* is photonegative toward strong light, photopositive toward weak light; it is probable that these animals become photopositive toward slightly greater illuminations with lower temperatures. This might play some part in determining movements into more illuminated areas during the winter season; presumably gonadic secretions might also be involved. Plessner ('13) came to the conclusion that the "eye-spots" of starfishes enable these creatures to respond to the *direction* of light; in *C. tenui-*

when the tide recedes. Since they are not strongly photopositive, they are seldom exposed to the drying action of the sun, and survive the temporary absence of water. Occasionally they have been found killed by rain (this has also been seen with *Stichopus mœbii*).

I always noticed that in the breeding months the proportion of animals which seemed to have recently undergone self division was much less than during the summer. Some attempt was therefore made to discover the percentage of recently divided specimens obtainable in each month of the year. This was done, somewhat unsystematically, for several years. The result is set forth in Table II. The data for each two-month period might have been averaged together, thus perhaps obliterating to some

TABLE II.

THE PERCENTAGE OF "NON-REGENERATING" COSCINASTERIAS SECURED IN RANDOM COLLECTIONS DURING EACH MONTH, 1915–1918.

Period.	Total No. of Individuals	Not Recently Divided,—*Per Cent.*
January	74	59
February	60	63
March	35	60
April	30	40
May	34	17
June	18	11
July	113	12
August	74	33
September	40	20
October	32	41
November	27	33
December	20	75

extent the possible variation from year to year. The numbers in the last column of the table express for each period the percentages in which the rays were 5 to 9 in number and of approximately equal length—in other words, those which exhibited no evidence of reasonably recent self division.

For June–July–August, 1913 and 1914, I found (Crozier, '15) 83.6 per cent. of the individuals to exhibit a group of longer and

spina, however, the tips of the rays shorten noticeably when a shadow is cast on them alone, whereas in sunlight the tips of the rays tend to be curled upward.

a group of shorter rays. In the same months of 1915–1918, the percentage was 80.4, a sufficiently good agreement. This is about the maximum percentage of regenerating, or recently divided, individuals encountered during a twelve-month period. From Fig. 2 it is evident that the highest percentage of intact specimens not recently divided occurred during December to March

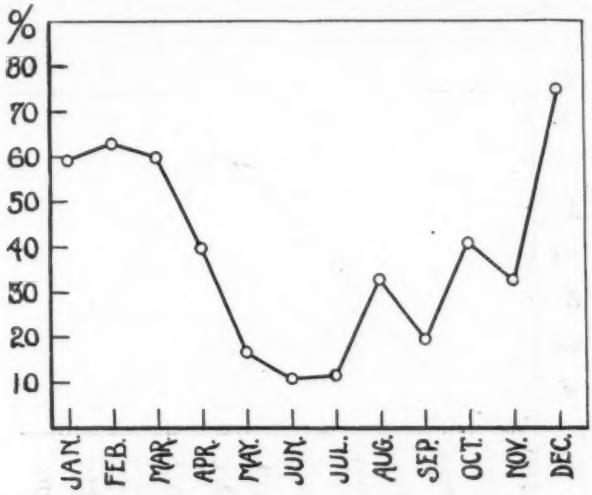

FIG. 2. The average percentage of *Coscinasterias* not showing evidence of recent self-division, during each month (1915–18).

—the percentage then rather rapidly sinking to a minimum during midsummer.

There is a certain correlation between the course of the curve of self-division (Fig. 2) and that of temperature-variation in the sea water. I reproduce here a seasonal temperature graph derived from figures given by Verrill (1902), together with the averages of my own data; the curves agree rather well, in spite of the fact that my temperature readings were not very numerous, nor systematically taken, but were obtained mainly for different special purposes having to do with other problems.

In comparing Figs. 2 and 3, it must be recalled that the "curve of self-division" is non-quantitative in one important respect: the percentages of dividing animals include data derived from indi-

viduals which may have divided some little time previous to others, as indicated by the various stages in regeneration classified under this head. A "lag" in this curve of division with respect to water temperature might therefore be expected. The sudden rise of percentage of "non-regenerating" specimens at December, however, is probably adequate proof that a month or so is sufficient time for the full growth of new rays. The actual "lag" in the curve of autotomy is of the wrong sort for the idea

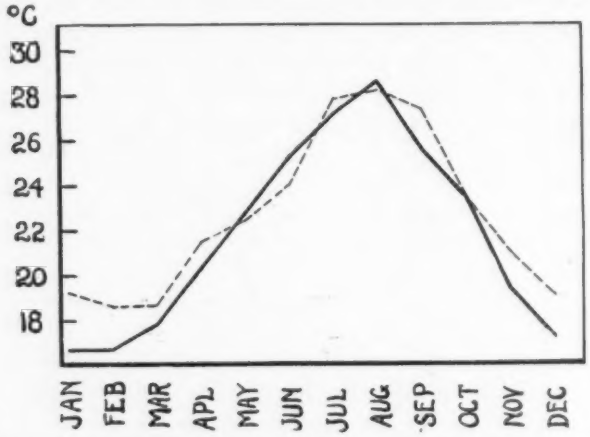

FIG. 3. Mean monthly temperatures of seawater at Bermuda. Heavy line, data from Verrill ('02); dotted line, averages from records of W. J. C. The dotted line records for winter months are higher than those given by Verrill, probably because my own observations were restricted for the most part to the waters of the partially enclosed Sounds.

of complete temperature control, because the decrease in frequency of self-division perceptibly precedes the seasonal decrease of temperature. This might be explained on the basis that the onset of higher temperature in spring serves indirectly to induce self-division, but that the capacity for this method of propagation is gradually exhausted, or in some fashion checked, before the warmer season has closed.

It is interesting, however, to notice how the frequency of self-division is enormously decreased at the approach of the period of breeding. There is thus accomplished, whether through the

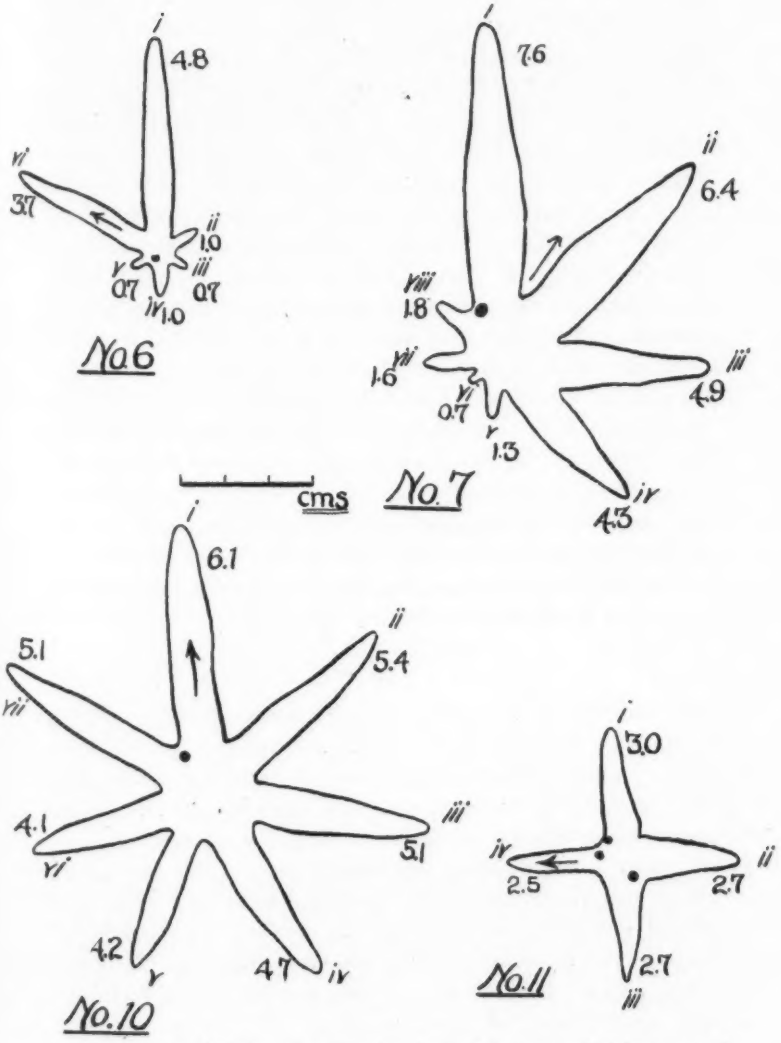

FIG. 4. The direction of progression (arrow) in two typical instances of recently autotomized halves of *Coscinasterias*. a, division surfaces; non-directive stimulation.

agency of temperature or in some other way, a nice adjustment of the two dissimilar methods of multiplication practiced by this species. Asexual increase through self-division of the body is at a minimum just before and during gametic reproduction. It is not difficult to suppose that an undivided individual is better suited for gamete formation and for such coördinated movements as probably are concerned in bringing the sexes near together for fecundation of the eggs. The general energy of the animal is then directed toward the elaboration of gametes, rather than diverted to the formation of new tissue. To what extent the elements of this correlative mechanism are causally related, however, one must refrain from guessing. Judging from the statements of Clark ('13*a*, '13*b*) and Mortensen ('17) regarding the asexual multiplication of *Linckia guildingii*, and the period of its egg-reproduction, I suspect that the supplementary relation here pointed out for *Coscinasterias* may not obtain with *Linckia*, where, however, the method of self-division is different, consisting not in the division of the body across the disc but in the abstriction of a portion of a ray. Nor in *Holothuria surinamensis* is there good evidence of an alternation of the sexual and asexual methods of multiplication (Crozier, '17).

II.

The number of madrepores in *Coscinasterias* is quite variable, from 1 to 5 being found on a disc. As earlier suggested (Crozier, '15), the number of madrepores is correlated rather definitely with the total number of rays (Tables III., IV.). It was observed that during the addition of new rays, subsequent to self-division, one or more new madrepores are also added. In almost every case, throughout the several hundred carefully examined, each moiety of a divided body bore one or several "old" madrepores. Moreover, some of the very largest specimens collected, with ray lengths of 12–13 cms., had but 5, 6, or 7 rays, of equal length, with but *one* madrepore. These facts suggested that the development of several madrepores is perhaps a more or less necessary preliminary to normal self-division. The madrepores are usually formed at widely separated points on the periphery of the disc; hence the probability is that each part of an

animal, when the creature divides, will carry at least one of these bodies.

TABLE III.

THE CORRELATION BETWEEN NUMBER OF MADREPORES AND NUMBER OF RAYS IN A RANDOM SAMPLE OF 275 COSCINASTERIAS.

Note that one or several madrepores are always present on the parts of recently bisected (autotomized) individuals.

MADEPORES.	NO. OF RAYS.									
	2	3	4	5	6	7	8	9	10	
1	2	5	4	5	13	21	10	2		62
2		2	4	5	10	25	14	3	2	65
3					6	29	30	2		67
4					5	18	28	4		55
5				1	1	2	4			8
	2	7	8	11	35	95	86	11	2	257

TABLE IV.

THE CORRELATION BETWEEN NUMBER OF MADREPORES AND NUMBER OF RAYS, AMONG 82 COSCINASTERIAS SELECTED SO AS TO INCLUDE ONLY INDIVIDUALS IN WHICH ALL THE RAYS WERE OF VERY NEARLY EQUAL LENGTH.

One or several madrepores commonly appears at the edge of the disc where rays are being newly added, subsequent to a self-division.

MADREPORES.	NO. OF RAYS.							
	3	4	5	6	7	8	9	
1	4	2	3	5	11	1	1	27
2	1	2	3	2	5	2	1	16
3		1		2	14	6		23
4				3	8	4	1	16
	5	5	6	12	38	13	3	82

Cole ('13) discovered that *Asterias forbesi* tends to creep in such a way that, in the absence of directive stimuli, the portion of the animal nearest to the single madrepore is usually in advance—a true, though subordinate, "physiological anterior." He suspected that the water-system might be concerned in some way with the determination of this "anterior." The conditions in *Coscinasterias* might perhaps be regarded in one, or both, of these two ways: (1) there is a physiological basis for the development of one or several madrepores according to the number

of rays, *i.e.*, depending on the total water requirements of the locomotor organs; or (2) the multiplicity of madrepores is adaptively related merely to the habit of self-division. At first I was inclined to entertain the former idea; the fact, however, that 7-, 8-, and 9-rayed individuals, as well as those of 5, 6, or 7 rays and of large size, do get along quite well with a single madrepore is more favorable to the second notion.

I would regard it as possible, then, that the multiplication of madrepores at separated points on the disc of *Coscinasterias* furnishes merely an assurance that portions of the body separated by autotomy will each be provided with a madreporic canal. It is not improbable, however, that the very development of supernumerary stone-canals, by furnishing each an additional "physiological anterior point,"—such as that revealed by Cole's ('13) experiments with *Asterias*,—provides automatically the very conditions determining self-division.

III.

The probability of this explanation of the significance of the madrepore might be tested by determining the direction of progression in various individuals, under non-directive stimulation. I studied from this standpoint the locomotion of 12 *Coscinasterias*. Non-directive conditions were secured very much as in Cole's ('13) experiments: an 8-c.p. filament, the only source of light, was suspended in the axis of a circular aquarium tub 3 feet in diameter, and 4 feet above the surface upon which the starfish crept. The tub held sea water to a depth of 10 cms. One half hour elapsed between trials. The observations were made at night. To horizontal light of this intensity, *Coscinasterias* was slightly photonegative, but more intense illumination was required to cause decided negative movements. Even under the supposition that illumination of a larger ray might lead to the determination of directed photonegative movements when the animal is illuminated vertically, owing to the larger sensory surface of such a ray, the conditions here established must be regarded as essentially non-directive, because the starfishes often crawled with some other than the longest ray *in advance*.

The length of the rays and the number of madrepores pre-

senting such great variations, it was possible to obtain a fairly definite idea of the relative values of *ray-length* and *madrepore-position* as determiners of the "physiological anterior"; for *Coscinasterias* does show definite tendencies in the orentation of its body with respect to creeping. Since the organization of the body of this starfish is somewhat irregular, the observations cannot be summarized in terms of morphologically defined rays (I did not note the position of the anal opening in these cases; cf. Gemmill, '14). I must therefore give several instances in detail (Table V.).

TABLE V.

SHOWING THE NUMBER OF TIMES EACH RAY OF THE STARFISHES ILLUSTRATED IN FIGS. 4 AND 5 WAS USED AS "DIRECTOR."

No. of the Animal.	No. of Trials.	Ray.								
		i.	ii.	iii.	iv.	v.	vi.	vii.	viii.	ix.
1	33	8	18	0	5	2				
2	24	10	10	1	1	1	1			
3	30	8	3	2	0	1	8	2	0	6
4	16	15	0	0	0	0	1			
6	16	1	0	0	3	0	12			
7	15	7	8	0	0	0				
10	16	8	0	0	0	0	4	4		

In the creeping of *Coscinasterias*, which is rather slow (at 24°, about 12–15 cms. per min.), several points are similar to ones emphasized by Cole in *Asterias*, notably the tendency to exhibit a definite region as "physiological anterior," with, however, clear evidence of the "rotation" of this anterior.[4] A good instance of such rotation is the following:

Coscinasterias No. 1, September 9, 1915. Creeping under vertical light, the region in advance shifted slowly, in this order (cf. Fig. 5): i, i, ii, ii–i, ii, ii, iii, iii–iv, v–iv, the animal being undisturbed from the outside.

Such "rotation of the impulse" was observed to be either clock- or anticlockwise. Ordinarily rotation of this sort was not

[4] Agersborg ('18), in describing the righting movements of the "twenty-rayed" *Pycnopodia helianthoides*, has spoken of "bilateral tendencies" in the activities of this starfish. But the condition of his tests are not well specified, nor is there any indication given of a relation between the "bilaterality" and structural conditions in *Pycnopodia*.

conspicuous, so that a fairly definite region could be distinguished as an "anterior" in creeping. From the typical records submitted, it appears that as a rule either the longest ray, or a long ray near a madrepore, is the "directive" ray.

As to the relative importance of ray-length and position of madrepore, it seems clear that, atlhough no absolute rule can be

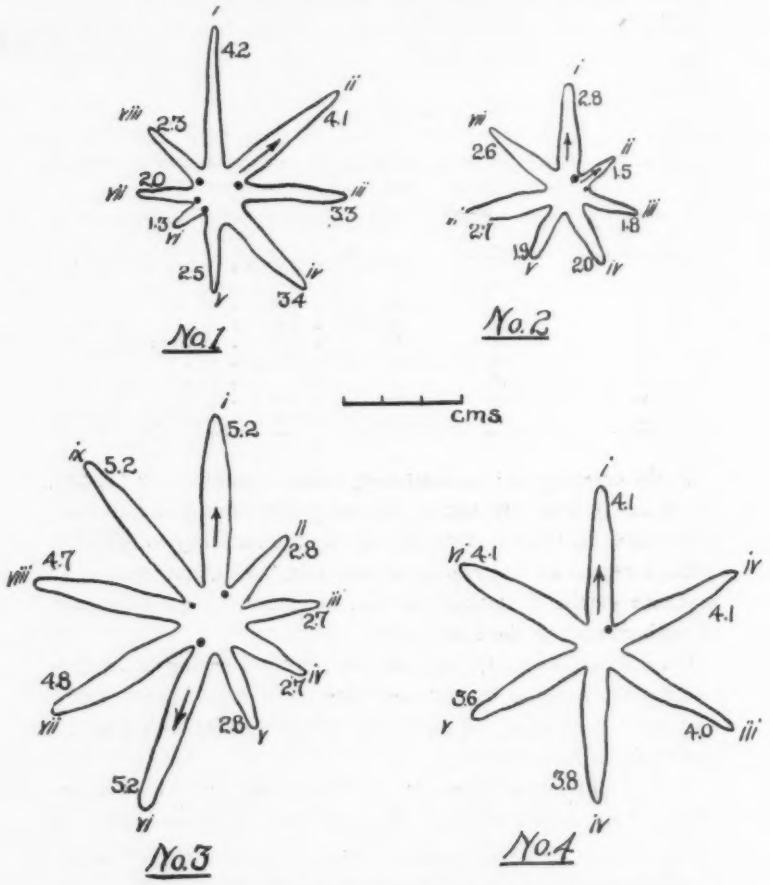

FIG. 5. Figs. 4 and 5 show typical instances of the "physiological anterior" in *Coscinasterias* (see Table V); *i*, *ii*, . . ., numbers assigned to rays; lengths of rays in cms.; madrepores, black; predominant directions of locomotion under non-directive stimulation shown by arrows.

stated, the latter factor is, on the whole, preponderant.[5] In 13 cases, where the disposition of rays and of madreporites was most favorable, the *longest ray* was recorded as "director," in the average, for 32.2 per cent. of the trials; a *ray near a madrepore* being so recorded, in the average, for 42.2 per cent. of the trials.

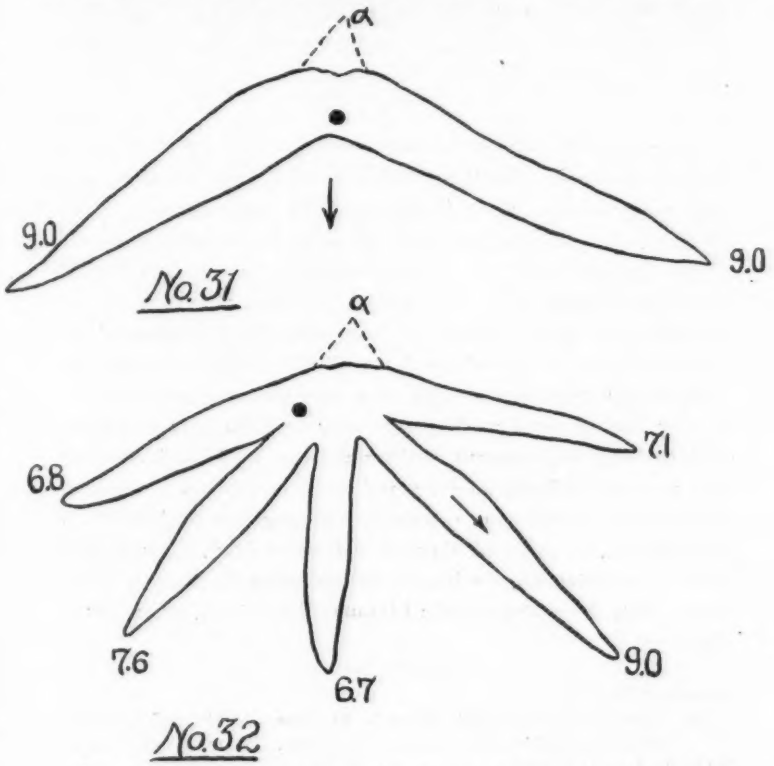

FIG. 6. The direction of progression (arrow) in two typical instances of recently autotomized halves of *Coscinasterias*. a, division surfaces; non-directive stimulation.

More convincing than such averages, however, are the actual data already set forth.

Frequently, in the case of animals having two ray-groups

[5] In connection with the matter of physiological and anatomical polarity in echinoderms, compare Clark's observations on the locomotion of *Comatula purpurea* ('15, p. 112), and mine on the creeping of *Mellita* (Crozier, '20); in both forms there is clear evidence of functional bilaterality.

greatly differing in size, the directive ray appears to be one next to the plane of antecedent autotomy. This fact might seem unfavorable to the conception of the "cause" of autotomy above formulated, but in fact I found that if the creeping of halves of starfishes recently separated was studied, the results were, on the contrary, favorable to this conception. Examples in point are afforded by such specimens as are illustrated in Fig. 6.

IV.

Summary.—It has been pointed out that in those individuals of *Coscinasterias tenuispina* which divided autotomously more than one madrepore is developed on the disc previous to division. Further, that each madrepore, or group of madrepores, is an external sign (at least) of the development of a tendency toward the establishment of a "physiological anterior" point, as expressed in creeping. Hence it is suggested that the cause of the autotomous separation of the body of this starfish into two approximately equal parts—each as a rule then automatically receiving one or more madrepores—may be intimately connected with the very development of these structures. It is shown that this asexual multiplication by self-division exhibits a seasonal rhythm so adjusted as to constitute a propagative method which supplements, in point of time as well as in kind, the ordinary gametic reproduction, the former method being distinctly in abeyance during the winter months (January–February), when breeding occurs.

CITATIONS.

Agersborg, H. P. K.

'18 Bilateral Tendencies and Habits in the Twenty-rayed Starfish, *Pycnopodia helianthoides* (Stimpson). BIOL. BULL., Vol. 35, pp. 232–254.

Clark, H. L.

'13a Autotomy in *Linckia*. Zool. Anz., Bd. 42, pp. 156–159.

'13b Investigations on Echinoderms at Montego Bay. Year Book 11, Carnegie Instn., Wash., pp. 134, 135.

'15 Commatulids of Torres Strait: with Special Reference to their Habits and Reactions. Publ. Carnegie Instn. Wash., No. 212, pp. 97–125.

Cole, L. J.

'13 Direction of Locomotion of the Starfish (*Asterias forbesi*). Jour. Exp. Zoöl., Vol. 14, pp. 1–32.

Crozier, W. J.

'15 On the Number of Rays in *Asterias tenuispina* Lamk. at Bermuda. Amer. Nat., Vol. 49, pp. 28–36.

'17 Multiplication by Fission in Holothurians. Amer. Nat., Vol. 51, pp. pp. 560–566.

'20 Notes on the Bionomics of *Mellita*. Amer. Nat., Vol. 54, pp. 435–442.

Gemmill, J. F.

'12 The development of the Starfish, *Solaster endeca* Forbes. Phil. Trans. Roy. Soc., B, Vol. 205, pp. 213–294.

'14 The Development and Certain Points in the Adult Structure of the Starfish, *Asterias rubens* L. Trans. Zoöl. Soc. Lond., Vol. 20, pp. 1–71.

Mortensen, T.

'17 On the Development of Some West Indian Echinoderms. Year Book 15, Carnegie Instn., Wash., pp. 193, 194.

Osterud, H. L.

'18 Preliminary Observations on the Development of *Leptasterias hexactis*. Publ. Puget Sd. Biol. Sta., Vol. 2, pp. 1–15.

Plessner, H.

'13 Untersuchungen über die Physiologie der Seesterne. I: Der Lichtsinn. Zool. Jahrb., Abt. Physiol., Bd. 33, pp. 361–386.

Verrill, A. E.

'02 The Bermuda Islands. x + 548 pp. New Haven.

NOTES ON SOME PROBLEMS OF ADAPTATION.

3. The Volume of Water Involved in the Cloacal Pumping of Holothurians (Stichopus).[1]

W. J. CROZIER,

Hull Zoölogical Laboratory, University of Chicago.

It has been of interest, especially in connection with studies of respiration, to determine the volumes of seawater which various marine animals pass through their respiratory organs. With the sponge *Spinosella* Parker ('14) calculated from his measurements that something like 78 liters a day was the volume forced through a single "finger" of the body of this sponge. For *Ascidia,* Hecht ('16) showed that the branchial stream was probably somewhat in excess of 173 liters per day, in individuals of medium size. The water current in such cases (the only ones of the kind carefully investigated) brings not only oxygen but food particles as well, and in addition has an excretory significance.

I undertook to measure the volume of seawater involved in the cloacal pumping of a holothurian such as *Stochopus mœbii,* in which water is forced into respiratory trees and then expelled to the exterior. The cloacal chamber of *Stichopus* pulsates with a frequency which depends on the size of the animal; after a certain number of pulsations the water contained in the respiratory trees is forced out; the number of pulsations between two acts of "spouting" also depends on the size of the individual (cf. Crozier, '16). As in all such cases, the expelled water is forced out rather violently, driven to a considerable distance, so that it is not readily taken in again when inspiration is again begun (Hecht, '16; Arey and Crozier, '19).

By previously ascertaining the mean number of inspiratory pulsations elapsing between "spoutings," it was possible to remove a given *Stochopus* from the water just before the beginning of an expiratory act, and to receive the discharged water in a funnel

[1] Contributions from the Bermuda Biological Station for Research. No. 122.

leading to a graduated cylinder. Employing 10 individuals each 24–25 cms. in length, I found an average volume of 15.5 c.c. of of seawater to be expelled in this way.

If a cut through the body-wall be made rapidly around the body of a *Stichopus* at the level of the anterior end of the cloacal chamber (Crozier, '16), it is possible to obtain a preparation in which the respiratory trees remain intact and preserve their normal connection with the cloacal wall. Such a preparation will live for a long time in seawater, and it can be seen that water is pumped, as normally, into the respiratory trees, and that when these organs have been expanded to a certain degree their contained water is expelled in the usual manner. In this process the respiratory trees themselves are actively contractile (cf. Iwanzoff, '97; Henri, '03); they shrivel up almost, but not quite, completely —so that nearly all their contained water is expelled. The independent nature of the activity of the cloaca and respiratory trees affords a curious instance of the autonomy of the organs in echinoderms.

Examination of the interior of an intact *Stichopus* at the conclusion of "spouting" showed that the respiratory trees were in this case also almost completely contracted.

The cloacal chamber of *Stichopus* 24.5 cm. in length pulsates with a frequency of 9.6 per minute (at 27°), and 8 or 9 inspiratory pulsations intervene between two expirations, each of the latter occupying some 12 seconds (cf. Crozier, '16). On this basis a *Stichopus* of this size will (at 27°) take into its cloaca about 15.5 c.c. of water during each 65 seconds, or about 859 c.c. per hour; leading to an estimated amount of 20–21 liters per day, assuming a uniform rate during the 24 hours.

From the comparative standpoint, it must be remembered that probably little or no food-getting is associated with the entrance of water into the body of *Stichopus* by way of the cloaca, Pütter's ('07) notion to the contrary notwithstanding; but that, in contrast to the situation in sponges and in ascidians, respiration and excretion are in holothurians the sole functional implications of the water stream. In this way it can be understood that the volume of water required by these animals is much less, even in proportion to their size, than is the case with sponges, lamellibranchs, or ascidians.

The respiratory value of the water moved by the cloacal pump is clearly evidenced by this fact: seawater in which a *Stichopus* was living had a reaction of pH 8.2; the pH of the water expelled during spouting was 7.8; that of the fluid within the body-cavity, 7.6. It is obvious that even within a minute's time relatively considerable quantities of CO_2 are able to diffuse across the thin membraneous wall of the distended respiratory trees. According to Winterstein ('09) about 50 per cent. of the respiratory loss of CO_2 is accomplished through the "trees."

CITATIONS.

Arey, L. B., and Crozier, W. J.
'19 The Sensory Responses of *Chiton*. Jour. Exp. Zoöl., Vol. 29, pp. 157–260.
Crozier, W. J.
'16 The Rhythmic Pulsation of the Cloaca of Holothurians. Ibid., Vol. 20, pp. 297–356.
Hecht, S.
'16 The Water Current Produced by *Ascidia atra* Lesueur. Ibid., pp. 429–434.
Henri, V.
'03 Étude des contractions rythmiques des vaisseux et du poumon aqueux chez les Holothuries. C. R. Soc. Biol. Paris, tom. 55, pp. 1314–1316.
Iwanzoff, N.
'97 Muskelelemente der Holothurien und ihr Verhalten zum Methylenblau. Arch. mikr. Anat., Bd. 49, pp. 103–113.
Parker, G. H.
'14 On the Strength and the Volume of the Water Current Produced by Sponges. Jour. Exp. Zoöl., Vol. 16, pp. 443–446.
Pütter, A.
'07 Die Ernährung der Wassertiere. Zeits. allg. Physiol., Bd. 16, pp. 65–114.
Winterstein, H.
'09 Über die Atmung der Holothurien. Arch. Fisiol., Vol. 7, pp. 33–40.

BIOLOGICAL STUDIES ON INTRACELLULAR BACTERIA.[1] NO. 1.

R. W. GLASER.

Introduction.

It has been known for a long time that certain blattids, homoptera, and ants harbor intracellular non-pathogenic organisms that are transmitted from generation to generation through the egg. Since Huxley's description of the pseudovitellus of aphids in 1858 a host of investigators have studied the embryonic development and morphology of this curious organ and its contents. After Huxley, the most prominent investigators associated with the study of this subject and with the development of the entire field of intracellular organisms in insects, were Lubbock, Metchnikoff, Balbiani, Krassilstschick, Blochmann, Lindner, Heymons, Berlese, Mercier, Pierantoni, Sûlc, Buchner and others.

This vast study has precipitated the following facts and theories which may be presented seriatim.

1. The insects that are definitely known to harbor intracellular non-pathogenic organisms belong to the family Blattidæ, the entire order of Homoptera, and the family Formicidæ.

2. It has also been reported that certain Lepidoptera and Coleoptera harbor intracellular non-pathogenic organisms, but conditions are not clear in the cases cited and the authors may have been confronted with true pathologic cases.

3. In the insects where non-pathogenic intracellular residents have been established one or more definite species of microorganisms are always associated with every individual of a particular species of host.

4. The microörganisms associated with the insects have been called symbionts.

5. The symbionts are transmitted from generation to generation through the egg in a very definite manner.

[1] Contribution from the Entomological Laboratories of the Bussey Institution, Harvard University. Bussey Institution No. 174.

6. In adult insects the symbionts are sometimes found free in the lymph, but in most species are found enclosed in very definite, modified cells known as mycetocytes or bacteriocytes.

7. In some species two or more mycetocytes fuse and become concentrated forming a very definite organ known as the mycetome. Some insects are monosymbiotic, that is, they harbor only one species of symbiont; others are disymbiotic or trisymbiotic.

9. When the host is monosymbiotic the symbionts may be found in mycetocytes or may be concentrated in a mycetome.

10. When the host is disymbiotic one species of symbiont may be found in mycetocytes and the other in a mycetome, or both symbionts in separate mycetomes or both symbionts in separate feebly connected mycetomes or lastly both species may be in one mycetome.

11. When the host is trisymbiotic we may have two species of symbionts in one mycetome and the third in mycetocytes or the three symbionts may be in one mycetome.

12. The host cells show specific changes, but no injury. They usually enlarge. If the symbionts are small the cytoplasm has the appearance of being finely reticulate; when large the cytoplasm becomes coarsely reticulate. The nuclei of the host cells are sometimes round; at other times curved or even arborescent. The organisms never invade the nuclei. In spite of deformation of the cytoplasm, the nucleus retains its ability to divide. Sometimes one finds nuclear and cytoplasmic degeneration, but this is an exception and probably due to some secondary parasite or to disease.

13. Most of the mycetocytes are enormous in size when compared with the remaining cells of the animal.

14. The systematic position of the symbionts is not always clear. In the case of the Blattids they are undoubtedly bacteria; in the case of some aphids undoubtedly yeasts, and in the other cases yeast-like organisms and probably higher fungi. No protozoan intracellular symbionts of insects are known.

15. The symbionts from a small number of host species have been cultivated upon artificial media, but in most cases the artificial cultivation experiments of symbionts have so far met with unsurmountable difficulties. This may be due to the fact that

the exact conditions existing in the mycetocytes or mycetome have not been sufficiently reproduced.

16. The number of symbionts per host is enormous. For *Ceroplastes rusci,* Berlese computed the number per host to lie between sixty and seventy thousand.

17. The reasons for the belief that the intracellular organisms are symbionts and not commensuals or parasites are the following:

(*a*) Every individual of a species is infected.

(*b*) The infection produces changes in the host cells, but these are harmless.

(*c*) The infection routes and methods of localization, while different in different hosts and symbionts, follow very definite courses within a species.

(*d*) The microörganisms are numerically controlled by the host, never increasing up to a point where they may prove fatal.

(*e*) The microörganisms within the insects obtain nourishment, and protection from drastic temperature and draught conditions.

18. While numerous theories have ben advanced, the symbiotic relationship, if any exists, has not been established upon any scientific basis.

Buchner (1912) in an extensive paper, together with original morphological investigations, gave an excellent review of all of the literature dealing with the subject of the intracellular symbionts of insects. For this reason, another extensive review of the literature would be merely a duplication of something that has already been admirably accomplished.

In beginning this investigation, I was primarily interested in the physiological aspect of the subject. If the intracellular organisms are really of some use to the host, they may possibly assist in the metabolism of reserve and other foods. This can only be accomplished through the secretion of enzymes by the microörganisms. I therefore thought it well to select one or two species of insects, to attempt to cultivate the intracellular organisms, then to make a systematic study of their enzyme activities, and later to attempt to correlate such activities with the metabolism of the host's diet.

For the above work, I chose two species of cockroaches for the reason that conditions seemed simpler. Cockroaches can be easily obtained and reared. They are large insects when compared with aphids, coccids, psyllids, aleurodids, etc., and can be readily dissected. Moreover, Mercier (1906) reported that he had cultivated the so-called "Blochmann bodies," thus putting an end to the tiresome discussions of Cuenot, Prénant and Henneguy, who supposed that the rods were metabolic products and not bacteria. Mercier cultivated these organisms on nutrient agar, gelatine, potato, milk and bouillon. He described only the morphological characters and named the organism *Bacillus cuenoti*. The fact that one species of cockroach had yielded an organism that could be cultivated also led me to choose these insects in preference to others. Lastly, cockroaches are monosymbiotic; in other words, conditions are not, so far as known, complicated by the presence of more than one microörganism.

Before proceeding to the cultural and physiological section of this paper it might be well to glance over the morphological results in Blattids as shown by Blochmann, Heymons, Buchner and others.

1. The Bacteriocytes and Bacteria in Blattids.

In *Blattela germanica* and *Periplaneta orientalis* certain of the fat cells are filled with bacteria measuring 6 to 8 μ long, straight or somewhat curved. The bacteria divide by binary fission. The cells occupied by the microörganisms do not contain any fat or urates and the nucleus is always normal.

The cockroaches present the bacteriocyte condition; there are no mycetomes nor complexes of mycetocytes nor, more strictly speaking in this case, bacteriocytes.

In *Blattela germanica* several rows of bacteriocytes are found in the fat body. The youngest eggs in the oviduct are free from infection. Somewhat older eggs, however, show several organisms on their surface. With the growth of the egg the number increases so much that later several layers surround the periphery. The direct wandering of the bacteria out of the host cells through the follicular membranes into the eggs, has not been ob-

served, but since the fat body surrounds the ovarioles, this is highly probable.

In *Periplaneta* bacteriocytes have been found in the oviduct. No bacteria have been noticed at the periphery of young oöcytes, but on observing large ones a number can be seen on the surface that have wandered out of the bacteriocytes, and must have made their way out through the follicular membrane. As the egg grows the bacteria become much more plentiful on the surface forming a closed layer around the entire egg. Soon the microorganisms become more concentrated at both poles and many are seen to lie perpendicular to the egg. In freshly laid eggs the bacteria are found within under the vitelline membrane, so they must have left the follicle, but the exact method has not been traced.

In Blattids generally, the later development takes place as follows: The organisms are concentrated in the middle of the yolk. The vitellophags also become infected. When the germ band grows around the yolk the bacteria get into the embryonic intestinal lumen. After a time they leave the lumen, wander through the intestinal epithelium and get into certain fat cells which they modify as previously shown. They take possession of the adipocytes and render them functionless as fat cells.

While the morphological details are not complete, they are very interesting. I found very similar conditions in the two species with which I worked, namely, *Periplaneta americana* and *Parcoblatta virginica.*

One of the most striking things in connection with this work is the definite wanderings of the microörganisms. They are motile forms in the insects studied by me, but that would not account for the prescribed course which they follow. I believe one must postulate a chemotropic response between the bacteria and certain host tissues in order to account for such behavior.

2. The Morphological, Cultural and Biochemical Characters of the Organisms Cultivated from Parcoblatta virginica and Periplaneta americana.

By dissecting cockroaches that portion of the fat body in which the bacteriocytes are embedded can be easily removed. It is

best to sacrifice one or two animals by exploration dissections, and by staining the excised tissue, in order to obtain an idea of the exact position of the desired cells and their microörganisms. When the region of the bacteriocytes has been discovered a fresh animal can be taken, etherized, pinned to a paraffin tray ventral side up, washed off with some sterilizing agent such as alcohol or a mixture of alcohol and corrosive sublimate and dissected with sterile instruments. The excised bacteriocytes with their contents can then be directly placed in the different sterile media and incubated.

The following are descriptions of the morphological, cultural and biochemical characters of the two species of organisms; one from *Parcoblatta virginica* and the other from *Periplaneta americana*. I consider it rather useless to give these bacteria specific names. To begin giving names to all of the thousands of symbionts, I think, would confuse rather than assist matters. It would be far better accurately to designate the host and then simply to refer to the genus of microörganism. In the case of disymbiotic and trisymbiotic insects one can refer to the two or three genera of symbionts associated with the host. If the two or three symbionts all belong to the same genus, the latter can be designated and the organisms labelled *a*, *b*, *c*, or 1, 2, 3.

The two species of organisms investigated by me are rather pleomorphic in cultures. Some individuals are straight like a bacillus; others are comma- or crescent-shaped like a spirillum. In the host cells the individuals are nearly all of the spirillum form. For this reason and owing to the fact that the organisms have one polar flagellum and do not form endospores, I am placing them in the genus *Spirillum*.

The organisms found by me in *Parcoblatta* and *Periplaneta* seem to differ from the morphological description of *Bacillus cuenoti* of Mercier. Since the cultural and biochemical characters of *B. cuenoti* were not described it has been rather difficult to make a comparison, however. The organism of Mercier seems to be a true bacillus, forming endospores of oval shape. The two organisms which I cultivated form no endospores.

Spirillum from Parcoblatta virginica.

Morphology.—1.5 per cent. neutral, nutrient agar, short and long rods. In nutrient bouillon longer rods, some curved, others straight. Average length 1.5–2 μ. Stains readily. Gram negative. No endospores. Motile. A single flagellum attached to one of the poles.

Nutrient Agar Stroke.—1.5 per cent. neutral. Growth: abundant, filiform, flat, glistening, smooth, opaque, butyrous. Odor absent. Medium greened.

Nutrient Bouillon.—Ring, clouding strong, odor absent, sediment abundant.

Gelatin Stab.—Growth best at top; line of puncture filiform. Liquefaction begins in two days, crateriform. Color of medium unchanged.

Litmus Milk.—Reaction not changed. Not coagulated. Reduction complete in eight days.

Milk.—Clearing without coagulation. Medium and consistency unchanged. No peptonization.

Potato.—Growth abundant, spreading, flat, glistening, smooth, butyrous. Odor absent. Medium unchanged.

Nutrient Agar Colonies.—1.5 per cent. neutral. Growth rapid at 35° C.; round, smooth, slightly convex, edge entire, amorphous. Diameter 1–1¾ mm.

Nutrient Gelatin Colonies.—Growth rapid, irregular, smooth, crateriform, edge undulate, liquefaction saucer.

Indol test negative.

NH_3 test faintly positive.

Nitrates not reduced to nitrites.

Diastase Test.—Starch converted to sugar.

Strong catalase reaction in bouillon culture.

Faint peroxidase reaction with gum guaiac and H_2O_2 in sugar bouillon culture.

FERMENTATION OF CARBOHYDRATES WITH FORMATION OF ACID AND GAS.

	Gas.	Acid.
Dextrose	0	0
Levulose	0	0
Saccharose	0	0
Maltose	0	0
Lactose	0	0
Mannit	0	0

Spirillum from Periplaneta americana.

Morphology.—1.5 per cent. neutral nutrient agar, short and long rods. Some curved, others straight. In nutrient bouillon, some straight rods and many curved. Average length 1.5–2 μ. Stains readily. Gram negative. No endospores. Motile. A single flagellum attached to one of the poles.

Nutrient Agar Stroke.—1.5 per cent. neutral. Growth abundant, filiform, flat, glistening, smooth, opaque, butyrus, odor absent, medium unchanged.

Nutrient Bouillon.—Ring, pellicle, clouding strong, odor absent, sediment abundant and flocculent.

Gelatin Stab.—Growth best at top, line of puncture filiform, liquefaction begins in two days, crateriform. Medium unchanged.

Litmus Milk.—No acid, coagulation prompt. Prompt reduction.

Milk.—Coagulation prompt, extrusion of whey. Consistency unchanged. Medium slightly greened. No peptonization.

Potato.—Growth scanty, slightly spreading, flat, dull, smooth, butyrous, odor absent, medium unchanged.

Nutrient Agar Colonies.—1.5 per cent. neutral. Growth rapid at 35° C., round, smooth, flat, edge entire, amorphous. Diameter ¾–2 mm.

Nutrient Gelatin Colonies.—Growth slow, colonies minute. Round, smooth, flat, edge entire, liquefaction saucer.

Indol test negative.

NH_3 test positive.

Nitrates not reduced to nitrites.

Diastase Test.—Starch converted to sugar.

Strong catalase reaction in bouillon cultures.

No peroxidase reaction.

FERMENTATION OF CARBOHYDRATES WITH FORMATION OF ACID AND GAS.

	Gas.	Acid.
Dextrose	o	o
Levulose	o	o
Saccharose	o	o
Maltose	o	o
Lactose	o	o
Mannit	o	o

It will be noticed from the foregoing descriptions that the morphological characters of the two species of spirilla are almost identical and if investigation were carried no further, one would doubtless consider the organisms from the two species of cockroaches as identical. Only in the cultural characters do the differences become striking and therefore I believe I am justified in considering the microörganisms as two distinct species. Taking Mercier's meager description of the symbiont from *Blatella* into consideration we should be inclined to assume that every living species of cockroach harbors a separate species of microorganism.

I did not test the pathogenicity of the two cultures on mammals. Perhaps this should have been done, since at present a great amount of interest centers on disease transmission by insects. Cockroaches are known to carry about and disseminate bacteria mechanically on their feet and in their intestines. Nevertheless, investigators interested in this matter might do well also to consider the intracellular organisms. Cockroaches are also in reality living reservoirs of thousands of bacteria enclosed within certain of the hosts' cells.

3. The Biochemical Activities of the Intracellular Organisms Studied.

First of all from the sugar tests it is interesting to note that the intracellular organisms do not ferment any of the sugars used. This is very fortunate for the insects. Undoubtedly sugar is present in some form taken in as food and if fermented by zymase secreted by the symbionts might anesthetize the animals with alcohol and CO_2.

The inverting enzyme sucrase is not produced by the *Parcoblatta* symbiont, but the *Periplaneta* organism inverts saccharose to levulose and dextrose.

Since the organisms are present in the fat body of adults and in the yolk of eggs, it seemed possible that they might have something to do with the metabolism of fat. Experiments with ethyl butyrate, milk fat and other fats failed to demonstrate the existence of lipase.

The cultural features demonstrated the fact that gelatin was

liquefied. In other words it was evident that the organisms secrete a protease. All proteins, however, are not hydrolyzed. For instance, casein does not seem to be effected for after the organisms have been grown in milk for the prescribed time the biuret test did not show the presence of propeptones or peptones.

It was found that both species of intracellular organisms produce diastase. The organisms were grown in sugar-free bouillon for ten days. After that time the cultures were poured into some pure potato starch paste with 2 per cent. thymol and incubated for 48 hours. Thereupon Fehling's solution was reduced showing that sugar had been formed. Check tests accompanied the experimental tests.

The tests for oxidizing enzymes really do not signify much, for they can be obtained with cultures of almost any bacterium such as *B. coli communis* or *B. prodigiosus*. However, both symbionts produce catalase and the *Parcoblatta* organism demonstrated the presence of peroxidase.

We may summarize the enzyme activities on the following table and then attempt to correlate some of them with the metabolism of the insects.

ENZYMES PRODUCED BY THE INTRACELLULAR ORGANISMS.

Symbionts.	Zymase.	Invertase.	Diastase.	Protease.	Lipase.	Catalase.	Peroxidase.
Parcoblatta organism	o	o	+	+	o	+	+
Periplaneta organism.....	o	+	+	+	o	+	o

It will be seen from the preceding table that the enzymes common to both species of symbionts are diastase, protease and catalase. From the standpoint of Blattid metabolism, I think, diastase and protease important. I was disappointed in not being able to demonstrate the existence of lipase, for conditions generally made me feel sure that there was an association between the microörganisms and fat metabolism.

Both species of insects studied feed primarily on carbohydrates (starches) and proteins, so it was encouraging to be able to demonstrate the two enzymes that effect these two classes of foods. Of course, the insects may feed on fats to some extent, but I think the statement that carbohydrates and proteins constitute their principal diet is fairly safe.

Philiptschenko (1907) studied the physiology of the fat body in *Blattela*. He did not mention the intracellular organisms, but found glycogen, proteins, fats and urates stored in great quantities especially in young animals.

From all of these facts, it is easy to assume that the symbionts assist in the carbohydrate and protein metabolism through enzyme secretion. Of course, the catalase may serve an oxidizing purpose, but I do not attach much weight to such a statement for the reason that protoplasm generally will give this as well as the peroxidase reaction in most cases. Nor do I lay any emphasis on the invertase found in the *Periplaneta* organism. It may serve a purpose, but this is not evident.

4. Conclusions.

In speaking of the intracellular organisms of insects, the word symbiont has been used rather freely in the literature. I have also made use of the word more for the sake of convenience than because I am convinced that we are confronted with true symbiosis. From the morphological studies presented by others, and from my own work it is a great temptation to refrain from thinking of a symbiotic relation. From my experiments it is easy to assume that the insect derives some benefit from the organisms as digestive organs, but what possible benefit can the microorganisms derive? Buchner's suggestions that the intracellular organisms are benefited by being protected within the insects from the drastic atmospheric influences of heat, cold, desiccation, etc., are a little far-fetched. Nor does Buchner's view that the organisms are also assured constant nourishment and opportunity to propagate appeal to me. Millions of bacteria and other organisms not found in the cells of animals manage to live and propagate just as well if not better. It is also necessary to remember that the insect tissue or serum must produce some powerful inhibitor in order to hold the propagation of the microörganisms within a reasonable limit, otherwise the host would be killed. The presence of an inhibitor is surely a handicap in the struggle for existence.

I think the interesting relations discussed had their origin in true parasitism, possibly, in disease. During an early period the

organisms now found as harmless entities within the insects were parasites producing pathologic conditions and disease. It seems to me, that such structures as the mycetocytes, bacteriocytes and mycetomes are a survival of previous profound pathologic changes. Later acquired immunity principles became heritable and the toxins and other effects came under control. The insects could not fully rid themselves of the invaders on account of the fact that transmission from generation to generation had established itself with unerring precision. Still later, the microorganisms lost all of their harmful effects and since they secreted a number of enzymes that proved serviceable to the hosts, the latter were not exterminated, but were probably diverted from their normal phylogenetic course. It is interesting to reflect what would have happened to those insects that harbor intracellular organisms if they had never been invaded by the parasites. If the hosts had not been able to overcome the infection they would have become extinct or at least reduced numerically to an appreciable extent. However, the insects were able to adapt themselves to profound changes and the phylogenetic history must have likewise taken another course. It is impossible to imagine that such structures as the pseudovitellus, mycetocytes and mycetomes with their inhabitants could have developed without altering the physiology and morphology and consequently the habits of the host.

The microörganisms may, as Buchner suggests, derive some benefit from the association, but this is not clear since they probably derived much more benefit as true parasites or disease producers. Judging from the ease with which the Blattid symbionts can be cultivated on almost anything, I feel sure that had they succeeded in exterminating all Blattids, they would still persist today living possibly as saprophytes under much more advantageous conditions.

LITERATURE CITED.

Balbiani.

'66 Note sur la reproduction et embryogénie des Pucerons. C. R. Acad. Sc. Paris, Vol. 62.

'75 Sur l'embryogénie de la Puce. C. R. Acad. Sc. Paris, Vol. 81.

Blochmann, F.

'87 Über das regelmässige Vorkommen von bakterienähnlichen Gebilden in den Geweben und Eiern verschiedener Insekten. Ztschr. Biol., Vol. 24 (N.F., Vol. 6).

'92 Über das Vorkommen von bakterienähnlichen Gebilden in den Geweben und Eiern verschiedener Insekten. Ctrbl. Bakteriol., Vol. 11.

Berlese, A.

'05 Sopra una nuova mucedinea parassita del *Ceroplastes Rusci*. Redia, Vol. 3.

Buchner. P.

'12 Studien an intracellularen symbionten. 1. Die intracellularen symbionten der Hemipteren. Arch. Protistenk., Vol. 26, Heft. 1.

Krassilschtschik, M.

'89 Sur les bactéries bioptytes. Note sur la symbiose des pucerons avec les bactéries. Ann. Inst. Pasteur, Vol. 3.

'90 Über eine Kategorie von Bacterien (Biophyten), die im Innern eines Organismus leben und ihm Nutzen bringen. 8. Kongress russ. Naturf. Ärzte. Sitz., 16. Jan., 1890. (Abstract in Bio. Ctrbl., Bd. 10, 1890.)

Lindner, P.

'95 Saccharomyces apiculatus parasiticus. Ctrbl. Bakteriol. Abt. 2, Vol. 1.

Mercier, L.

'06 Les corps bactérioides de la Blatte (*Periplaneta orientalis*). C. R. Soc. Biol. Paris, Vol. 61.

'07 Recherches sur les bactérioides des Blattides. Arch. Protistenk., Vol. 9. Cellules à Bacillus cuenoti dans la Paroi des Gaines ovariques de la Blatte. C. R. Soc. Biol. Paris, Vol. 62.

Philiptschenko.

'07 Concerning the fat-body of the black cockroach (*Stylopyga orientalis*). Russian. Revue Russe Entomol., Nr. 4.

Pierantoni, U.

Ulteriori osservazioni sulla simbiosi ereditaria degli Omotteri. Zool. Anz., Vol. 36.

Sûlc, K.

'06 Kermincola Kermesina N. G. N. Sp. und Physokermina N. Sp., neue Mikroendosymbiontiker der Cocciden. S. B. Böhm. Ges. Wiss. Prag.

'10 "Pseudovitellus" und ähnliche Gewebe der Homopteren sind Wohnstätten symbiotischer Saccharomyceten. Ibid.

'10 Symbiotische Saccharomyceten der echten Cicaden. Ibid.